IN THE UNITED STATES DISTRICT COURT
FOR THE SOUTHERN DISTRICT OF GEORGIA
SAVANNAH DIVISION

CHRIS JAZAIRI, Plaintiff. VS. ROYAL OAKS APARTMENT ASSOCIATES, L.P., Its Parent Company And Subsidiaries, And MITCHELL L. MORGAN MANAGEMENT, INC. Defendants,	)	CIVIL ACTION FILE NO.: CV-04-404-091

PLAINTIFF'S FIRST MOTION IN LIMINE
RE: RAYMOND HARBISON, PH.D.

COMES NOW Plaintiff and moves to exclude the expert testimony of Defendant, including testimony offered by Raymond Harbison, Ph.D., based on the lack or relevancy and lack of reliability of the testimony and in support of this motion states the following:

1. Defendant has named Dr. Raymond Harbison as an expert witness in the above referenced action. Dr. Harbison has no personal knowledge of any facts in this lawsuit. See Harbison Report attached hereto as Exhibit 1. Dr. Harbison is a pharmacologist/toxicologist and he has submitted an extensive resume on the many pharmacological studies in which he has participated. None of these studies involved the effects of mold. See Harbison C.V. attached hereto as Exhibit 2 at pp. 14-48. Dr. Harbison has not published any peer-reviewed studies on the allergic effects of molds on humans. Dr. Harbison is not qualified to comment on the allergic effects of mold on humans.

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2. Dr Harbison is not a medical doctor. Exhibit 2. Dr. Harbison is not licensed in any state to render a diagnosis or treatment to any person for illness, disease or injury. As a matter of law, he is not qualified to examine, diagnose or treat a person. He cannot prescribe medication or testing. He is not licensed to interpret medical objective studies, including x-rays, blood tests or pulmonary function tests in determining the illness of a person. In forming his opinions, Dr. Harbison has relied on medical records of medical doctors who have examined, diagnosed and treated Plaintiff. Exhibit 1 at p. 1. Dr. Harbison is not qualified to comment on the cause of Plaintiff's injury.

3. Additionally, Dr. Harbison is not legally qualified to comment on whether Plaintiff has suffered any problems associated with allergies. Dr. Harbison has not published any studies on the cause of allergic reactions to molds or in a damp environment. He has not ever legally examined, diagnosed or treated a person for an allergic condition. As a matter of law, Dr. Harbison is not allowed to examine, diagnose or treat anyone for an allergic lung condition or any other type of lung condition. Dr. Harbison is not qualified to comment on whether Plaintiff's lung condition is related to her exposure in apartment 1607, Royal Oaks Apartments.

4. Dr. Harbison's mistaken belief about Plaintiff's medical condition is demonstrated in his expert statement when he refers to Plaintiff's lung fibrosis which requires years to develop and, therefore, could not have developed while she was in the apartment. Harbison Report, Exhibit 1 at p. 7, ¶ 3. Not a single treating medical doctor has contended that Plaintiff has lung fibrosis which developed over a long period of years. Dr. Harbison does not recognize the symptoms of hypersensitivity pneumonitis. However, Dr. Harbison steps into the shoes of the treating medical doctors to opine on the medical cause of Plaintiff's illness.

5. Dr. Harbison also presumes that Plaintiff's injuries are the result of a toxic exposure, and, therefore, he reasons that Plaintiff cannot have had a toxic exposure because the scientific community has not established a dose response relationship to specific molds. This presumption is demonstrated by Dr. Harbison statement about the minimal standards that are necessary to establish a cause and effect relationship for "toxic effects" which include the need for "cytotoxicity". Exhibit 1 at Harbison Statement at p. 5 at items d, e and h. Dr. Harbison then states that because there is no evidence of a dose relationship with toxic levels of mold and mycotoxins, Plaintiff could not have been injured while living in the apartment. Id. at p. 5. However, Plaintiff's doctor, Dr. Johanning, has diagnosed Plaintiff with an inflammatory allergic reaction akin to hypersensitivity pneumonitis. Johanning Depo. at 73-74, 111-112, attached as Exhibit 3. The medical literature requires exposure but does not require a specific threshold dose before a diagnosis can be made of this allergic condition. Exhibit 4, 5, 6, and 7. There is no known dose response relationship established by medical science relevant to an allergic reaction, as distinguished from a toxic reaction. Exhibit 8. Dr. Harbison's opinions about the necessity of a dose response relationship to establish a toxic reaction is irrelevant.

6. Dr. Harbison is not an industrial hygienist. Exhibit 2. Dr. Harbison does not collect or analyze mold samples in the regular course of his practice. He does not inspect residences for unhealthy dampness or elevated levels of mold in the usual course of his practice. Defendant has not presented any evidence that Dr. Harbison has any qualifications to comment on Ken Warren's evaluation of the mold exposure of Plaintiff in Apartment 1607, Royal Oaks Apartment and the need for remediation as confirmed by the Chatham County Department of Health. Dr. Harbison's comments on Mr. Warren's evaluation are outside of Dr. Harbison's area of expertise.

7. Dr. Harbison is not a mycologist. Exhibit 2. Defendant has not presented any evidence that Dr. Harbison is qualified to testify on the nature of molds. He is not qualified to determine the most accurate method of evaluating a residence for mold exposure. Additionally, Dr. Harbison is not qualified to comment on the most probable level of mold spores in the apartment. Additionally, any comments on the nature of molds, particularly the molds found in apartment 1607, Royal Oaks Apartment would be outside Dr. Harbison's expertise.

8. Dr. Harbison would be qualified to testify on pharmacological or toxicological testing, but such testing is irrelevant to the allegations in this lawsuit. Exhibit 2.

9. The expert report of Dr. Harbison is required to be a complete statement of his opinions and rationales. Rule 26(a)(2)(B). Any testimony from Dr. Harbison must be limited to his opinions and rationales stated in his report. Id.

WHEREFORE, Plaintiff respectfully requests that the testimony of Dr. Harbison be limited in conformance with the trial court's gatekeeping function set forth in Daubert v. Merrell Dow Pharm. Inc., 509 U.S. 579 (1993) and the notice requirements of the federal rules of civil procedure. Rule 26(a)(2)(B), F.R. Civ. P.

THIS, the 29 day of April, 2005.

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IN THE UNITED STATES DISTRICT COURT
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CHRIS JAZAIRI, Plaintiff. VS. ROYAL OAKS APARTMENT ASSOCIATES, L.P., Its Parent Company And Subsidiaries, And MITCHELL L. MORGAN MANAGEMENT, INC. Defendants,	)))))))))	CIVIL ACTION FILE NO.: CV-04-404-091

BRIEF IN SUPPORT OF
PLAINTIFF'S FIRST MOTION IN LIMINE
RE. RAYMOND HARBISON, PH.D.

I. STATEMENT OF THE CASE

Plaintiff Chris Jazairi, a tenant in apartment 1607 of Royal Oaks Apartment in Savannah, from May until the end of August of 2002 has been diagnosed with an allergic inflammatory lung condition resulting from exposure to an unhealthy damp residential living environment that included atypical elevated levels of mold. Plaintiff has sued the landlord in tort for recovery for her injury.

II. ARGUMENT AND CITATION OF AUTHORITY

A. The Federal Rules of Evidence Limit Expert Testimony

Defendant has proffered the testimony of Dr. Harbison on a broad range of topics including medical causation, exposure levels and the nature of molds. Dr. Harbison is a

pharmacologist/toxicologist. Dr. Harbison's testimony should be limited because (1) he is not qualified to testify competently regarding the matters he intends to address; (2) his proffered expert testimony is unreliable under Rule 702; (3) his proffered expert testimony is irrelevant under Rule 402; and (4) his proffered expert testimony is unfairly prejudicial under Rule 403.

The Federal Rules of Evidence provide for the admission of expert testimony when "scientific, technical, or other specialized knowledge will assist the trier of fact." Fed. R. Evid. 702. Under Rule 702, in such situations a witness "qualified as an expert by knowledge, skill, experience, training, or education may testify . . . in the form of an opinion or otherwise." Id.

The Supreme Court in Daubert v. Merrell Dow Pharm., Inc., 509 U.S. 579 (1993), focused on the admissibility of scientific expert testimony, finding that such testimony is admissible only if the expert is competent to present it, and it is both reliable and relevant. The Court explained that "Federal Rule of Evidence 702 allows the admission of expert testimony only if: (1) the expert is competent and qualified to testify regarding the matters that he intends to address; (2) the methodology by which the expert reaches his conclusions is sufficiently reliable; and (3) the expert, through scientific, technical or specialized expertise, provides testimony that assists the trier of fact to understand the evidence or determine a fact in issue." Siharath v. Sandoz Pharm. Corp., 131 F. Supp. 2d 1347, 1351 (N.D. Ga. 2001) (citing Daubert, 509 U.S. at 590-91); Allison v. McGhan Med. Corp., 184 F.3d 1300, 1309 (11th Cir. 1999); City of Tuscaloosa v. Harcros Chemicals, Inc., 158 F.3d 548, 562 (11th Cir. 1998)). To ensure that these elements are present, the Court held that, under Rule 702, trial judges are to serve as the "gatekeeper" to the introduction of such evidence. Daubert, 509 U.S. at 591-93. "The judge's role is to keep unreliable and irrelevant information from the jury because of its inability to assist in the factual determinations, its potential to create

confusion, and its lack of probative value." Allison, 184 F.3d at 1311-12.

"The burden of laying the proper foundation for the admission of the expert testimony is on the party offering the expert, and admissibility must be shown by a preponderance of the evidence." Id. at 1306. "Where the burden has not been satisfied, Federal Rule of Evidence 702 precludes expert testimony." *Siharath*, 131 F. Supp. 2d at 1351.

In performing its "gatekeeping" function, the trial court is required, under Federal Rule of Evidence 104(a), to answer preliminary questions regarding the admissibility of expert testimony under Rule 702, examining the expert's opinion testimony for competence, reliability, and relevance. The first element addresses whether "the expert is qualified to testify competently regarding the matters he intends to address." Allison, 184 F.3d at 1306. Under Rule 702, the question is whether the witness is qualified as an expert "by knowledge, skill, experience, training or education" in the area concerning which he or she will present expert testimony. Fed. R. Evid. 702. The court must examine "not the qualifications of a witness in the abstract, but whether those qualifications provide a foundation for a witness to answer a specific question." Berry v. City of Detroit, 25 F.3d 1342, 1351 (6th Cir. 1994), cert. denied, 513 U.S. 1111 (1995). If the witness is not qualified to testify competently regarding the matters he or she intends to address, then his or her testimony should be excluded. See United States v. Paul, 175 F.3d 906, 912 (11th Cir. 1999) (witness's review of literature in area outside his field "did not make him any more qualified to testify as an expert . . . than a lay person who read the same articles"); City of Tuscaloosa, 158 F.3d at 563 ("[P]ortions of [plaintiffs' expert's] testimony lie outside of his competence as a statistician . . ., thus requiring the exclusion of those portions of [his] data and testimony . . . .").

If the Court determines that the witness is qualified to testify competently regarding the

matters he or she intends to address, the Court must next assess the reliability of the proffered expert testimony. The Court must determine "whether an expert's testimony reflects 'scientific knowledge,' whether the findings are 'derived by the scientific method,' and whether the work product amounts to 'good science.'" Daubert v. Merrell Dow Pharm., Inc., on remand, 43 F.3d 1311, 1316 (9th Cir. 1995), cert. denied, 516 U.S. 869 (1995) (quoting Daubert, 509 U.S. at 590, 593)). The adjective "scientific" in "scientific knowledge" implies "a grounding in the methods and procedures of science. Similarly, the word 'knowledge' connotes more than subjective belief or unsupported speculation." Daubert, 509 U.S. at 590. Thus, "[u]nder the regime of Daubert . . . a district judge asked to admit scientific evidence must determine whether the evidence is genuinely scientific, as distinct from being unscientific speculation offered by a genuine scientist." Allison, 184 F.3d at 1316-17 (quoting Rosen v. Ciba-Geigy Corp., 78 F.3d 316, 318 (7th Cir. 1996)). See also Cartwright v. Home Depot U.S.A., Inc., 936 F. Supp. 900, 905 (M.D. Fla 1996) ("Even experts must show that they used science and not speculation to come to their conclusions."). The burden of proving the reliability of proffered expert testimony rests with the person seeking to use it; "the expert's bald assurance of validity is not enough." Daubert, 43 F.3d at 1316.

B. Dr. Harbison's Proffered Testimony Does Not "Fit" Under *Daubert*

"The final element of admissibility, set forth in Daubert, is an appropriate relevance, or 'fit,' between the expert's opinion and the facts of the case." Siharath, 131 F. Supp. 2d at 1352 (citing Daubert, 509 U.S. at 591; United States v. Gilliard, 133 F.3d 809, 812 (11th Cir. 1998); United States v. Smith, 122 F.3d 1355, 1358-59 (11th Cir. 1997)). "'Fit' is not always obvious, and

scientific validity for one purpose is not necessarily scientific validity for other, unrelated purposes." Daubert, 509 U.S. at 591. "Scientific testimony does not assist the trier of fact unless the testimony has a valid scientific connection to the pertinent inquiry." Siharath, 131 F. Supp. 2d at 1352 (citing Daubert, 509 U.S. at 591). "There is no 'fit' where there is 'simply too great an analytical gap between the data and the opinion offered,' as when an expert offers animal studies showing one type of cancer in laboratory mice to support causation of another type of cancer in humans." Id. (citing General Elec. Co. v. Joiner, 522 U.S. 136, 146 (1997)).

In the case sub judice, Dr. Harbison's opinion about toxic mold injury does not "fit" into the facts of the case. Toxic injury from mold is not an issue. The dose-response relationship between toxic exposure and human injury is irrelevant to the case sub judice. Plaintiff has not been diagnosed with her injury toxic exposure.

Plaintiff has suffered an allergic lung inflammation. Dr. Johanning, a medical doctor, has applied medical science in diagnosing Plaintiff with allergic inflammatory response. Johanning Depo. at 54, 74, 126-27 at Exhibit 3. Dr. Harbison is not a medical doctor and is unqualified to criticize medical science because he is not a medical doctor. Dr. Harbison has no qualifications to establish the standard necessary for a medical diagnosis. Furthermore, Dr. Harbison's opinions stated in his written report on the cause and effect of toxins is irrelevant. Daubert v. Merrell Dow Pharm. Inc., 509 U.S. 579 (1993).

C. Dr. Harbison Is Unreliable Because He Is Not Qualified to Testify Competently Regarding the Matters He Intends to Address

Dr. Harbison intends to express general opinions at trial on a broad range of issues which are

outside of his expertise. For instance, his expert report addresses issues of medical diagnosis and practice. While Dr. Harbison may be qualified to opine generally about the ability of specific toxins he has studied to cause injury to humans, he is not qualified to comment on Plaintiff's medical condition or the cause of her condition. Dr. Harbison has also not examined or treated Plaintiff. Dr. Harbison's opinion on the medical standard that must be satisfied to establish a medical diagnosis must be excluded. Daubert, 509 U.S. at 579.

Dr. Harbison's expert report addresses Plaintiff's medical condition and he relies on his review of medical records in forming his opinion. However, he is not qualified to make such statements as a matter of law. O.C.G.A. § 43-34-26. He does not have a medical license to diagnose or treat anyone, no matter what their condition. For instance, Georgia strictly limits the practice of medicine to license medical doctors. O.C.G.A. § 43-34-26. Dr. Harbison is not a medical doctor. His testimony on medical practice and diagnosis would be unreliable. Therefore, he can not comment on Plaintiff's illness or the cause of it. Daubert, 509 U.S. at 588-95.

Dr. Harbison is no more qualified to testify competently regarding the cause of Plaintiff's medical condition and the cause of her condition than a lay witness who read her medical records. See Paul, 175 F3d at 912; City of Tuscaloosa, 158 F3d at 563. Because Dr. Harbison is not qualified to testify competently regarding the matters he intends to address at trial, his opinion on the cause of Plaintiff's illness should be excluded under Rule 702. Daubert, 509 U.S. at 588-95.

Additionally, Dr. Harbison does not have the qualifications to comment on Mr. Warren's opinion which is based on the practice of industrial hygiene. Mr. Warren inspects and advises property owners and insurance companies on the need for mold remediation based on this opinions of potential unhealthy mold exposure as a regular part of this business. See Warren Affidavit, App.

Vol. I at Exhibit 31, attached hereto as Exhibit 9. Dr. Harbison does not have this experience or expertise. While Dr. Harbison is certainly bright and accomplished, his area of expertise in pharmacology and toxic exposures would not qualify him to perform tasks outside of his field. Daubert, 509 U.S. at 588-95l; Paul, 175 F.3d at 912.

Additionally, Dr. Harbison is not qualified to opine on the nature of molds. He does not study molds. He is not a mycologist. He has not published on the field of mycology. He has apparently picked up a book which discusses molds and is reciting what a book says. Unfortunately, he has misunderstood or mischaracterized the book's statements. See Horner Affidavit attached hereto as Exhibit 11 at ¶¶ 6-7. Dr. Harbison is no more qualified to speak about molds than a layman. See Paul, 175 F3d at 912; City of Tuscaloosa, 158 F3d at 563.

D. Dr. Harbison's Testimony Should Be Limited Under Rule 402 Because It Is Irrelevant

Dr. Harbison's proffered testimony on the need for a dose response study to establish the toxic effects of each of the molds that may have been in Plaintiff's apartment should also be excluded because it is irrelevant. Rule 402, F.R.E. Plaintiff's lung condition was either caused by an allergic reaction, as testified to by Dr. Johanning [Johanning Depo at pp. 126-27, Exhibit 3], or by cigarette smoking, as suspected by Dr. Costanzo. Costanzo Depo. at 42, Exhibit 10. No treating doctor contends that Plaintiff has suffered a toxic reaction to mold that has caused longstanding lung fibrosis. Therefore, Dr. Harbison's testimony about the lack of any studies on the dose response relationship of molds is irrelevant. Daubert, 509 U.S. at 588-95.

F. Dr. Harbison's Testimony Must Be Limited to Opinions and the Basis for Those Opinions in His Expert Report

The federal rules require that a specially retained expert provide a full statement of his opinions and the basis and reasons therefrom, all materials that he relied upon in forming his opinions and all exhibits he will refer to in support of his opinions. Rule 26 (a)(2)(B), F.R.Civ.P. Dr. Harbison has written a 9 page report and Plaintiff has relied on this report as a full statement of his opinions. The committee that commented on the federal rules contemplated that the reports might eliminate the need for depositions. *See* Rule 26 F.R.E. Committee Notes, 1993 Amendments ¶ (2).

Dr. Harbison should not be allowed to extend his testimony beyond the opinions and rationales for those opinions that are stated in his expert report. Dr. Harbison's testimony should be limited only the opinions that are properly supported and are not cumulative in his expert report. Rule 26(a)(2)(B) F.R. Civ. P.

F. Dr. Harbison's Testimony Should Be Limited under Rule 403

Rule 403 may serve as an additional basis for excluding expert testimony that, although relevant, has probative value which is "substantially outweighed by the danger of unfair prejudice, confusion of the issues, or misleading the jury, or by considerations of undue delay, waste of time, or needless presentation of cumulative evidence." Fed. R. Evid. 403. "The Supreme Court recognized in Daubert the intricate role of Rule 403 in an expert testimony admissibility analysis when it noted that expert testimony could be 'both powerful and quite misleading because of the

difficulty in evaluating it.'" Allison, 184 F.3d at 1310 (quoting Daubert, 509 U.S. at 595). "Thus, while Rules 401 and 402 reflect the general policy of the Federal Rules for liberal admission of evidence, Rule 403, working in conjunction with Rules 702 and 703, militates against this general policy by giving courts discretion to preclude expert testimony unless it passes more stringent standards of reliability and relevance. These stricter standards are necessary because of the potential impact on the jury of expert testimony." Id.

In light of the many infirmities of Dr. Harbison's expert statement in this case, whatever minimal probative value his opinions might have would be substantially outweighed by the danger of unfair prejudice to Plaintiff or of misleading the jury. Therefore, Dr. Harbison's testimony should be strictly limited under Rule 403.

CONCLUSION

An Order is attached for the Court's review. Plaintiff respectfully requests that this order be entered which strictly limits Dr. Harbison's testimony only to matters in which he is qualified to opine, which are relevant to this case and which he properly disclosed.

THIS, the 29 day of April, 2005.

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ORDER ON PLAINTIFF'S FIRST MOTION IN LIMINE

Having read and considered Plaintiff's First Motion in Limine, it is hereby granted. Dr. Harbison's testimony will be limited to his area of expertise in pharmacology and toxicology. Dr. Harbison is not a medical doctor and is not qualified to discuss or render any expert opinion on the symptoms, diagnosis, treatment or cause of Plaintiff's medical condition. Therefore, Dr. Harbison may not testify on her symptoms, diagnosis, treatment or cause of Plaintiff's alleged injuries. Daubert v. Merrell Dow Pharm. Inc., 509 U.S. 579 (1993)

Furthermore, Dr. Harbison is not an industrial hygienist and does not inspect, test or remediate properties for mold or other moisture related problems as a regular practice. Therefore, Dr. Harbison is not qualified to comment on the evaluation of apartment 1607, Royal Oaks Apartments by Ken Warren, an industrial hygienist, or by the Chatham County Department of Health. Therefore, Dr. Harbison may not testify on the methods of evaluating the apartment, on the degree of mold contamination, on moisture-related damage in the apartment or on the healthy or unhealthy environment in the apartment. Id.

Furthermore, Dr. Harbison is not a mycologist and does not study the nature of molds. Also, he does not collect mold samples, analyze mold samples or interpret mold samples as a regular part of his practice. Therefore, Dr. Harbison may not testify on the nature of molds, including those found in the apartment, on the methods of collection and analysis of the mold samples or on the interpretation of the mold analysis. Id.

Furthermore, the testimony of Dr. Harbison is limited to the statements and opinions which he has described in his expert report of December 6, 2004. Rule 26(a)(2)(B), F.R.Civ.P.

So Ordered this _____ day of _____, 2005.

_____
Hon. B. Avant Edenfield
Judge, U.S. District Court
Southern District of Georgia, Savannah

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REPORT OF DR. RAYMOND D. HARBISON

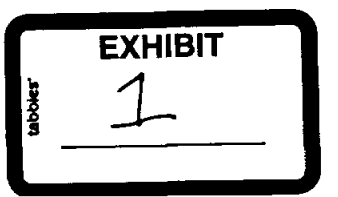
I am a board certified toxicologist and Director of the Center for Environmental/Occupational Risk Analysis and Management at the University of South Florida, Tampa, Florida. I am also a Professor of Environmental and Occupational Health in the College of Public Health at the University of South Florida, Professor of Pharmacology and Pathology in the College of Medicine at the University of South Florida, and adjunct Professor of Medicine at the College of Medicine, State University of New York at Buffalo. I earned a doctorate in Pharmacology/Toxicology at the University of Iowa, College of Medicine in 1969. I served on the faculty of Tulane Medical School, New Orleans, Louisiana from 1969 to 1971 and Vanderbilt Medical Center, Nashville, Tennessee from 1971 to 1981. I served as Director of the Interdisciplinary Toxicology Program at the University of Arkansas for Medical Sciences, Little Rock, Arkansas from 1981 to 1988. I was a Professor of Pathology, Toxicology and Pharmacology at the Health Science Center at the University of Florida, Gainesville, Florida from 1988 to 1996.

My experience includes research and teaching related to general toxicology, human health risk assessment, evaluating potential mechanisms of toxicity in association with alleged health effects, and evaluating patients potentially affected by chemical exposures who present to the Occupational and Environmental Medicine Clinic at the University of South Florida.

I have served on the editorial boards of scientific journals, including *Fundamental and Applied Toxicology, Environmental Health Sciences, Teratogenicity, Carcinogenicity, and Mutagenicity,* and *Research Communications in Pharmacology and Toxicology*. I have served on advisory committees for the National Academy of Sciences, National Research Council, American Cancer Society, the United States Environmental Protection Agency, the National Institute of Occupational Safety and Health, National Institute of Environmental Health Sciences, and the National Institute for Drug Abuse.

I have authored over 150 scientific articles, and have published several chapters in textbooks of industrial hygiene and occupational medicine. I have published a textbook of industrial toxicology. My membership in scientific societies includes the Society of Toxicology, Society for Risk Analysis, Teratology Society, American Society for Pharmacology and Experimental Therapeutics, and the New York Academy of Sciences. My curriculum vitae, which is attached as Exhibit "1," includes a complete list of my publications.

I have reviewed materials that include but are not limited to the deposition testimony of the Plaintiff Chris Jazairi and her medical records, the complaint filed by the Plaintiff, initial disclosures and interrogatory responses by the Plaintiff, the Report of Douglas R. Haney, the Report of Kenneth R. Warren, visual inspection information and photographs for Unit 1607 of the Royal Oaks Apartments in Savannah, Georgia, and a report (7-29-02) from the Chatham County Department of Public Health and results of samples taken August 2, 2002 from Unit 1607, sampling results of Dr. Richard Lipsey, rough draft deposition testimony of Dr. Eckardt Johanning, and other materials.



I understand Chris Jazairi claims that mold discovered in her residence of Unit 1607 Royal Oaks Apartments, Savannah, Georgia caused and/or contributed to a variety of her current health conditions and complaints.

From my training and experience, I am thoroughly familiar with the scientific and medical literature concerning mold and mycotoxins. I am also familiar with the documented effects of mold and mold mycotoxins on humans.

In addition, from my training and experience, I am aware of available scientific literature describing the scientific methodology required to determine the cause of a human disease or ailment. I am aware of the requirement to apply this same methodology to establish the causal link between a mold exposure and alleged diseases and conditions, which would occur spontaneously or by chance, from those which may be caused as a result of mold and mycotoxin exposure. If the scientific methodology is not followed, arbitrary and incorrect associations may be made because of individual bias, confounders, and failure to use a precise and objective comparison.

I have reviewed the opinions and conclusions of Mr. Doug Haney, Mr. Kenneth Warren, and Dr. Eckardt Johanning, clinical laboratory testing results, medical records, residence testing results and other information, and records from other health care providers concerning the complaints and conditions of Chris Jazairi.

In any situation involving environmental exposure to mold and mycotoxins, where the alleged exposure is followed by an allegedly observable physiological change or deficit, the ultimate problem confronting the practitioner attempting to attribute a cause lies in determining whether there is a basis for concluding that the observed effect would not have occurred in the absence of mold and mycotoxin exposure.

I have reviewed the residential history of Chris Jazairi. I am aware that Mr. Doug Haney and Mr. Kenneth Warren are alleging that mold and mycotoxin exposure in her residence either could have caused or did cause her current conditions and complaints. I understand Dr. Eckardt Johanning opines the initial complaints of Chris Jazairi were caused by mold and dampness at Unit 1607, but these effects have resolved and are not associated with her current complaints.

It is my conclusion after reviewing these materials that the methodology and procedures used by Mr. Doug Haney and Mr. Kenneth Warren to reach their conclusions that Chris Jazairi was exposed to a harmful concentration of mold and/or mold toxins, or that any of this exposure caused or contributed to her current conditions, are not based on valid and reliable scientific principles and are not generally accepted by the scientific community. In addition, the data relied upon and advanced as supportive of their opinions is not based on valid and reliable scientific principles and is not generally accepted by the scientific community as supplying an adequate basis for the conclusions reached. Moreover, none of the records of other medical providers support a conclusion that Ms. Jazairi's alleged health conditions were caused by exposure to mold at the Royal Oaks Apartments. In sum, proof is lacking that Chris Jazairi either was exposed to a harmful amount of mold or mycotoxin in her residence or that any

residential mold or mycotoxin exposure could have or, in fact did, cause her present conditions and complaints.

In fact, there are many published conclusions that do not support the opinions of Mr. Doug Haney and Mr. Kenneth Warren and the claims of Chris Jazairi. For example, the American College of Occupational and Environmental Medicine Council on Scientific Affairs (2002) published that,

> **"Current scientific evidence does not support the proposition that human health has been adversely affected by inhaled mycotoxins in home, school, or office environments"**

In addition, Dr. Harriet Burge of the Harvard School of Public Health published (2001) that,

> **"The fact that a mold is growing in a home is not good evidence for exposure of any kind, and certainly not evidence of any danger."**

Further, the American Industrial Hygiene Association (2001) published that,

> **"Ultimately, the panel concluded that at this time there is not enough evidence to support an association between mycotoxin fungi and a change in the spectrum of illness, the severity of illness or an increase in illness."**

Page and Trout (2001) of the National Institute for Occupational Safety and Health published that,

> **"This review of the literature indicates that there is inadequate evidence to support the conclusion that exposure to mycotoxins in the indoor (nonindustrial) environment is causally related to symptoms or illness among building occupants."**

Other published literature indicates that,

> **"Although exposure to molds can produce significant mucosal irritation, there are very few data to suggest long-term ill effects. More importantly, there is no evidence in humans that mold exposure leads to nonmucosal pathology..."(Assouline-Dayan et al., 2002)**
>
> **"specific toxicity due to inhaled fungal toxins has not been scientifically established" (Fung and Hughson, 2002)**

"Specific toxicity due to inhaled mycotoxins is not well documented, and remains controversial." (Fung and Hughson, 2002)

"Adverse health effects from inhalation of *Stachybotrys* spores in water-damaged buildings is not supported by available peer-reviewed reports in medical literature." (Texas Medical Association, 2002)

Finally and most recently, the Institute of Medicine, of The National Academies concluded that there was inadequate or insufficient evidence to determine whether an association exists for possible mold effects such as,

Dyspnea (shortness of breath)
Asthma development
Airflow development
Mucous membrane irritation syndrome
Chronic obstruction
Inhalation fevers
Lower respiratory
Acute idiopathic pulmonary hemorrhage in infants
Skin symptoms
Gastrointestinal tract problems
Fatigue
Neuropsychiatric symptoms
Cancer
Reproductive effects
Rheumatologic and other immune diseases

(Institute of Medicine, Damp Indoor Spaces and Health, 2004)

Various individuals have attempted to devise a methodology sufficient for linking disease processes occurring in a specific individual following specific exposures. There is general agreement that in the absence of certain information, no valid conclusion as to cause and effect can be made in a specific individual's case.

The following minimal information is required by the scientific community before any reasonable medical or scientific probability can be expressed relating an exposure to an observed effect in a specific individual.

a. Exposure to a putative agent must be documented.

b. The exposure must occur in such a fashion that the substance is temporally eligible to be the cause of the observed effect.

c. The exposure level must be documented at a level capable of inducing a known toxic effect.

d. The observed toxic effect, whether acting directly on the target organ, or indirectly through alteration of body chemistry or function must be satisfactorily linked to the observed effect in the target organ. The observed effect must be biologically plausible and known to be caused by the agent.

e. A toxic effect suspected of being responsible, either directly or indirectly for injury to a specific organ must have been replicated in a general population upon identical exposure.

f. Confounding variables, such as drug-induced, intrinsic factors, or effects caused by infectious diseases, must be eliminated as potential causal or contributing factors.

g. If the latency period (the time between exposure and alleged effect) is extended, some plausible explanation for delay of onset of the disease process must be present, either through data from similarly exposed populations, or other sources.

h. The specific effect from the putative agent must be demonstrated as occurring in the specific individual involved. In cases where no effect can be demonstrated other than injury to a target organ, no conclusion can be drawn unless specific cytotoxicity affecting the target organ can be demonstrated.

i. A consistent pattern of identical effects under controlled circumstances must be demonstrated (literature precedence).

j. A consistent morphologic pattern under controlled circumstances (or a pathognomonic effect) must be demonstrated and existence of the specific morphologic pattern confirmed in the individual case under consideration.

k. Epidemiologic and bioassay tests must be supportive.

Neither Mr. Doug Haney nor Mr. Kenneth Warren have met these minimum requirements.

There is no objective evidence that Chris Jazairi was exposed to harmful or toxic levels of mold and mycotoxins while living in the residence at Unit 1607 at Royal Oaks Apartments. Regardless of that fact, none of the other minimal requirements generally accepted by the scientific community as being required before a causal relationship can be attributed in a specific individual's case are present here. However, Mr. Doug Haney and Mr. Kenneth Warren rely on the belief that exposure to mold and mycotoxins as described in the various reports for the residence can cause the current complaints and conditions of Chris Jazairi. This conclusion is not

consistent with scientific and medical literature and public policy. The information available provides a "snapshot" look at the mold present in the residential environment at the time of visual observation and sampling. This does not identify and characterize the actual level of any residential exposure of Chris Jazairi to mold and mycotoxins. Further, there are no federal or state standards that identify harmful concentrations of mold and mycotoxins in indoor environments. Generally, indoor levels of mold are compared to outdoor levels of mold to determine whether there is an "amplification site" for mold growth indoors. But even reports of indoor levels of mold exceeding outdoor levels fall well short of the required evidence for identifying a purported harmful dose of mold and mold toxins that a person received.

Dr. Richard Lipsey sampled personal belongings of Chris Jazairi more than nine months after she moved out of Unit 1607 at Royal Oaks Apartments. These sampling results cannot be extrapolated to the residence of Chris Jazairi because they were removed and stored elsewhere. Further, these sampling results do not justify the destruction of approximately $90,000. of personal property. These sampling results cannot be used to reliably determine the mold content of Chris Jazairi's personal property while living in Unit 1607.

A move-in punch list for Unit 1607 notes mold in the second bath and a letter to Royal Oaks Apartments indicates visible mold on air vents in upstairs bathrooms and on walls around air vents. The mere presence of mold does not result in mold and mycotoxins entering the body. Exposure is only the opportunity for contact. The presence of mold does not always result in airborne spores. For example, *Stachybotrys* spores do not easily enter the air because of the sticky nature of this mold growth. Exposure does not necessarily result in mold or a putative agent entering the body. A visual inspection by the Chatham County Department of Public Health identified mold in bath laundry, master bedroom, around tub, and air-conditioning vent. Sampling by Chatham County Department of Public Health (8-2-02) identified mold in two bulk samples from the lower and upper bath. Various fungi were found in the bulk samples. The reliability of these samples is suspect, because the samples were obtained August 2, 2002 but were not received and analyzed by the laboratory until more than a month later. However no air samples were collected and analyzed for viable and non-viable fungi. There are no samples that indicate air levels of viable and non-viable mold exceeded any levels known to cause lung injury. The average indoor air concentration of Colony Forming Units (CFU) per cubic meter (m^3) of air reported in the scientific literature for non-complaint residential structures was 1,252 CFU/m^3. There is no sampling data that indicates the air levels in Unit 1607 ever exceeded that level. The range reported in the scientific literature of indoor air concentrations of spores per cubic meter of air was 68 to 2,703 spores/m^3. Again, there is no sampling data that indicates the air levels in Unit 1607 ever exceeded these levels. Further, seasonal outdoor spore concentrations varied from 543 to 5,423 spores/m^3, for example, during September 2001 through December 2001 in Charlotte, NC. Therefore, airborne fungus exposure is normal and there is no data that indicates exposure in Unit 1607 was distinguishable from outdoor, non-complaint, and normal residences. Further, no indoor air surveys identified any mycotoxins present in the indoor air of Chris Jazairi's residence. Therefore, any claim that Chris Jazairi was exposed to mycotoxins resulting from mold growth in her residence is speculation.

These sampling results likewise cannot be used to justify the further remediation of Unit 1607 as proposed by Mr. Kenneth Warren. There is no objective validation of the efficacy of the

additional remediation of Unit 1607 proposed by Mr. Kenneth Warren. There is no evidence that the proposed remediation would significantly alter any exposure of Chris Jazairi to mold and mycotoxins.

Mr. Doug Haney and Mr. Kenneth Warren contend that mold and mycotoxin exposure in Unit 1607 were excessive and could cause adverse health effects. However, neither Mr. Haney nor Mr. Warren has relied on any air measurements of mold or mycotoxins for their opinions. Their opinions are based on extrapolation and their methodology for extrapolation has not been disclosed nor validated. Further, the error rate of their extrapolation methodology is not known. They have not used any reliable methodology to arrive at their conclusions that there were mold and mycotoxin levels in the air of Unit 1607 that were sufficient to cause the complaints and conditions of Chris Jazairi.

There has not been a specific mold or mold toxin-induced toxic effect, attributable to mold from the residence at Unit 1607 Royal Oaks Apartments that has been shown to be the cause of the present complaints and conditions of Chris Jazairi and no specific mold and/or mold toxin-induced cytotoxicity has been demonstrated. The present condition of lung fibrosis is not temporally eligible to be caused by the alleged exposure at Unit 1607. Development of lung fibrosis takes years and could not have been produced in a few weeks or months as a result of an alleged exposure at Unit 1607. Such a claim is not biologically plausible.

No empirical data is available to indicate that the residential mold exposure identified in the various reports for the Unit 1607 residence of Chris Jazairi is capable of inducing her present conditions. No direct or indirect evidence, indicating that a dose of mold and/or mycotoxin resulting from the Unit 1607 residence is capable of altering body tissues, has been provided. Neither Mr. Doug Haney nor Mr. Kenneth Warren have provided any evidence that the alleged household mold exposure of Chris Jazairi could have resulted in a dose sufficient to cause her current conditions and complaints. No specific exposure analysis is provided for Chris Jazairi. They rely simply on the history provided by Chris Jazairi and the limited sampling and reports as the exposure basis for the conclusion that mold, mycotoxins, and the residential environment at Unit 1607 is the cause of the present conditions and complaints of Chris Jazairi. This methodology is not consistent with a scientific methodology and these opinions are not concordant with the scientific and medical literature.

None of the residential visual inspection and sampling reports establish that Chris Jazairi received a harmful dose of any mold or mycotoxin.

None of the clinical laboratory test results establish that Chris Jazairi received a harmful dose of any mold or mycotoxin from the indoor environment of Unit 1607 Royal Oaks Apartments, Savannah, Georgia. Chris Jazairi was not found to be allergic to mold and mold was not identified in her respiratory system. Although Dr. Eckardt Johanning opines that the initial complaints of Chris Jazairi were due to an allergic response to mold in her residence, there is no evidence to support that opinion. Dr. Johanning agrees that the present complaints of Chris Jazairi were not caused by mold and conditions at Unit 1607. However, he has not used a reliable methodology for concluding the initial complaints of Chris Jazairi were caused by mold and damp conditions at Unit 1607. He also has not determined the frequency, duration, and level of

exposure of Chris Jazairi to a putative agent. The rate of error of his methodology is not known. Further, he has not properly ruled out smoking and other possible causes of her initial complaints nor properly ruled in mold as a cause of her initial complaints. Finally, contrary to Dr. Johanning's opinion, the Institute of Medicine concluded that there is inadequate or insufficient evidence to determine an association between lower respiratory illness in adults and damp indoor environment.

No consistent pattern of similar effects as seen in Chris Jazairi under controlled circumstances has been reported in the scientific literature. There are no reliable epidemiological studies that support a cause and effect relationship between the mold and mycotoxin exposure claimed by Chris Jazairi and her complaints and conditions. Further, there are no reliable epidemiological studies that support the opinions and conclusions of Mr. Doug Haney, Mr. Kenneth Warren, and Dr. Eckardt Johanning.

Chris Jazairi is 39 years old and has a history of two decades of smoking a pack of cigarettes a day and consumes approximately four ounces of alcohol on a daily basis. She also lived with a smoker in Unit 1607. She lived in the Unit for about four months. Her current condition and complaints are similar to when living in Unit 1607 even though she has not lived in Unit 1607 for more than two years. One of her health care providers, Patricia Costanzo, MD (7-20-04) noted,

> **"Chris moved to the mid-west and then back here. She is headstrong about the mold issue. She has seen several other doctors in regard to it. I am not sure if she wants me to help her and treat her or whether she simply wants me to try to help her with a lawsuit. " (MED0031)**

> **"She feels tired and short of breath on exertion. She has anterior chest pain or tightness. She has a slew of other complaints referable to almost every body system, all of which she is trying to blame on the mold." (MED0031)**

> **"However, the results of my bronchoscopy failed to recover any mold" (MED0031)**

Further, neither Mr. Doug Haney, Mr. Kenneth Warren, nor Dr. Eckardt Johanning has provided comparative risk analyses for the smoking and drinking history of Chris Jazairi. These are risk factors for her present condition and complaints.

Absent reliable epidemiological studies that support a cause and effect relationship between mold exposure and her complaints and conditions and evidence that Chris Jazairi received a harmful dose of mold and mycotoxin from the indoor environment of the residence at Unit 1607, any testimony by Mr. Doug Haney, Mr. Kenneth Warren, and Dr. Eckardt Johanning alleging Chris Jazairi has suffered injuries as a result of exposure to mold and mycotoxins at the dwelling is speculation.

Based upon a review of all of the exposure evidence and the present conditions and complaints of Chris Jazairi, it is my opinion that the methodology and procedures adopted by Mr. Doug Haney, Mr. Kenneth Warren, and Dr. Eckardt Johanning are not based on valid and reliable scientific principles, have not been adequately tested, are not generally accepted by the scientific community, and when subjected to peer review, have been rejected by the scientific community.

In addition, the scientific basis that is relied upon is wholly inadequate. The evidence relied upon by Mr. Doug Haney, Mr. Kenneth Warren, and Dr. Eckardt Johanning can fairly be described as non-existent. They have provided no reliable evidence of a harmful mold and mycotoxin exposure resulting from residing at Unit 1607. They have relied on the belief that exposure to mold, based on the description of Chris Jazairi and the various reports provided, caused the present condition and complaints of Chris Jazairi. Even under the most minimal standards, the evidence is totally absent to draw a conclusion that household mold exposure as described by Chris Jazairi and others and sampled by Chatham County Department of Public Health caused or contributed to the present conditions and complaints of Chris Jazairi.

No reasonable practitioner would conclude that sufficient evidence exists for linking this household mold exposure, as identified by Chris Jazairi and various reports, with the alleged conditions and complaints of Chris Jazairi based on the facts of this case.

I reserve the right to change and/or supplement my opinion should additional information become available.

Raymond D. Harbison

RAYMOND D. HARBISON, M.S, Ph.D.

12-06-04

Center for
Environmental/Occupational
Risk Analysis & Management

COLLEGE OF PUBLIC HEALTH
UNIVERSITY OF SOUTH FLORIDA

OCCUPATIONAL, ENVIRONMENTAL & AGRICULTURAL DISEASE CONTROL & PREVENTION

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Professor & Director

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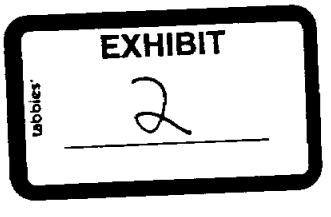

CURRICULUM VITAE

Raymond D. Harbison, M.S., Ph.D.
Center for Environmental/Occupational Risk Analysis & Management
College of Public Health
13201 Bruce B. Downs Blvd.
Tampa, Florida 33612-3805

Home Address: 15445 51st Drive
Wellborn, Florida 32094

Date and Place of Birth: January 1, 1943, Peru, Illinois

EDUCATION

Drake University, Des Moines, Iowa - 1961 to 1965, B.S.

University of Iowa, Iowa City, Iowa - 1965 to 1967, M.S. (Pharmacology) -
Thesis: Induced hyperbilirubinemia and a quantitative method or analysis of diazotized bilirubin.

University of Iowa, College of Medicine, Iowa City, Iowa - 1967 to 1969, Ph.D. (Pharmacology/Toxicology) - Dissertation: Studies on the mechanism of teratogenic action and neonatal pharmacology of diphenylhydantoin.

SUMMARY OF EXPERIENCE

2004-PRESENT
DEPARTMENT OF HEALTH
STATE OF FLORIDA
ENVIRONMENTAL PUBLIC HEALTH TRACKING ADVISORT COMMITTEE

2003-PRESENT
ASSOCIATE MEMBER
H. LEE MOFFITT CANCER CEMTER & RESEARCH INSTITUTE
TAMPA, FL

2003-PRESENT
CHAIRMAN, PUBLIC HEALTH OVERSIGHT COMMITTEE
SAUDI ARABIA

2002-PRESENT
RESEARCH PROFESSOR, Center for Environmental Diagnostics and Bioremediation
The University of West Florida
Pensacola, FL

2001-PRESENT
TRAINING DIRECTOR, NIOSH ,HAZARDOUS SUBSTANCES CONTINUING EDUCATION TRAINING
NIOSH - EDUCATION AND RESEARCH CENTER, UNIVERSITY OF SOUTH FLORIDA

2000-PRESENT
BROWNFIELD ADVISORY BOARD- HILLSBOROUGH COUNTY, FLORIDA

2000-Present
Editorial Board- Research Communications in Pharmacology and Toxicology

1999-Present
Vice Chair, Technical Advisory Committee, Brownfield Advisory Board, City of Clearwater, Florida

1998-Present
Toxicologist, Research Service, Haley Veterans Administration Hospital, Tampa, Florida

1998-Present
Professor, Department of Pathology, College of Medicine, University of South Florida

1995-Present
Adjunct Professor, Department of Medicine, College of Medicine, State University of New York at Buffalo

1995-Present
Professor, Department of Pharmacology and Therapeutics, College of Medicine, University of South Florida

1995-Present
Professor, Department of Environmental and Occupational Health, College of Public Health, University of South Florida

1995-Present
Director, Center for Environmental/Occupational Risk Analysis & Management, College of Public Health, University of South Florida

1996-Present
Director, Certification of Hazardous Materials Handlers Review Course and Examination

1994-Present
National Institute of Health, Reviewers Reserve

1994-1995
Advisory Board of Center for Training, Research, and Education for Environmental Occupations (TREEO), University of Florida

1993-1997
American Cancer Society Study Review Group (Florida)

1993-1997
Academy of Toxicological Sciences, Board of Directors

1992-1997
Advisory Board of Earth 2020, the University of Virginia's Center for Environmental Policy

1991-1995
Faculty, Superfund University Training Institutes, East Tennessee State University and University of Virginia

1991-1995
Science Advisory Board Consultant, Environmental Health Committee, United States Environmental Protection Agency

1990-1995
Professor, Department of Pathology, College of Medicine, University of Florida

1989-1995
Director, Laboratory for Environmental and Human Toxicology, University of Florida

1989-1997
Speciality Staff, St. Vincent Infirmary Medical Center, Little Rock, Arkansas

1989-1996
Board of Advisors, The Environmental Institute

1989 - 1994
National Institute of Drug Abuse, Pharmacology II Study Review Group

1988-1995
Professor, Department of Pharmacology and Therapeutics, College of Medicine, University of Florida

1988-1995
Professor, Department of Physiological Sciences, Health Science Center, University of Florida

1987-1997
Clinical Professor, Department of Preventive Medicine, Medical College of Wisconsin, Milwaukee, Wisconsin

1986-1990
National Institute of Environmental Health Sciences Study Review Group

1984-1985
Chairman, National Institute of Occupational Safety and Health Study Review Group

1982-1986
Editorial Board, Fundamental and Applied Toxicology

1980-1990
Society of Toxicology Liaison with Teratology Society

1980-1988
Professor, Department of Pharmacology, University of Arkansas for Medical Sciences

1980-1984
National Institute of Occupational Safety and Health Study Review Committee

1979-Present
Editorial Board, Teratogenicity, Mutagenicity, Carcinogenicity

1977-1980
Director, Toxic Substance Control Laboratory and Associate Professor, Department of Pharmacology and Biochemistry, School of Medicine, Vanderbilt University, Nashville, Tennessee

1977-1980
Professional Affairs Committee - American Society for Pharmacology and Experimental Therapeutics

1977-1980
National Institute on Drug Abuse - Review of DAWN (Drug Abuse Warning Network)

1977-1979
Graduate Education Committee, Vanderbilt University

1977-1978
National Research Council Committee to Review Scientific Program of National Center for Toxicological Research

1976-1980
Editorial Board, Environmental Health Sciences

1976-1977
National Academy of Science, Advisory Center for Toxicology - Revision of Toxicity Testing Procedures for Consumer Protection Agency

1975-1976
Technical Committee of the Society of Toxicology, Chairman

1975-1976
National Institute on Drug Abuse Center Review Committee

1974-1980
Editorial Board, International Journal of Addictive Diseases

1974-1979
Vanderbilt Medical Center, Animal Care Committee

1974-1978
Consultant, U.S. Congressional Committee on Safety Assessment of Chemical Additives & Drugs

1974-1975
Standing Policy Committee on Biomedical Sciences, Vanderbilt School of Medicine; National Institute on Drug Abuse, Clinical Behavioral Review Committee

1974-1975
Co-Chairman, Technical Committee of the Society of Toxicology

1972-1976
Assistant Professor of Pharmacology and Biochemistry, Vanderbilt University School of Medicine

1971-1975
National Institutes of Mental Health-Narcotic Addiction and Drug Abuse Review Committee, Biomedical-Pharmacology-Toxicology

1971-1972
Assistant Professor, Department of Pharmacology, Tulane Medical School

1971-1972
Director of Teratology Section, Laboratory of Environmental Health, Department of Medicine, School of Medicine, Tulane University

1969-1970
Instructor of Pharmacology, Tulane Medical School

1965-1969
USPHS Trainee, University of Iowa, Department of Pharmacology, College of Medicine, Iowa City, Iowa

PROFESSIONAL SOCIETIES

Rho Chi Honorary Pharmacy Society
Sigma Xi (Promotion of Research in Science)
American Association for the Advancement of Science
Teratology Society
Society of Toxicology
American Society for Pharmacology and Experimental Therapeutics
New York Academy of Science
Society for Risk Analysis

AWARDS
1978
Society of Toxicology Achievement Award

CERTIFICATION
1982
1987
1992
1997
2002
Certified in General Toxicology
Registration
Registered Professional Industrial Hygienest
1999

INDUSTRIAL EXPERIENCE

- Occidental Oil - Worker safety in oil shale production
- Shell Development Corporation - Pesticide use and safety
- Petrolite Corporation - Health assessment of waste incineration methods
- Monsanto Corporation - Chemical mutagenesis and the workplace, environmental assessment of PCB pollution
- American Academy of Industrial Medicine - Women in the workplace
- Tennessee Occupational Safety Health Administration - Industrial toxicology training course
- Society Organic Chemical Manufacturing Association - Chemical carcinogenesis
- Sanitary Corporation of America - Worker safety at industrial residue landfill sites
- Ethyl Corporation - Chemical-induced mutagenesis and teratogenesis in the workplace
- State of Kentucky Bureau of Natural Resources - Toxicology training course for solid waste management personnel
- U.S. Environmental Protection Agency - Chemistry and toxicology of hazardous materials training course for spill management personnel
- Ecology and Environment, Inc. - Health and safety program advisor
- Texaco - Toxicology consultant
- Hooker Chemical Company - Evaluation of health effects at Love Canal
- Chemical Manufacturers Association - Technical review of health effects of PCBs
- American Petroleum Institute - Comments to EPA concerning Resource Conservation Recovery Act
- IBM Corporation - Reproductive hazard assessment
- U.S. Environmental Protection Agency - Rebuttable presumption review of pesticides (FIFRA)

- United States Department of Agriculture - Review of aerial pesticide applications
- State of Georgia Department of Environmental Protection - Toxicology training course for emergency environmental incident management
- State of North Carolina Environmental Resource Management Division - Toxicology training course for environmental management and public health personnel
- Exxon - Review of teratogenic hazard of benzene exposure
- U.S. Department of Justice - Evaluation of environmental and public health problems associated with Price Landfill, Atlantic City, New Jersey
- U.S. Department of Justice - Evaluation of environmental and public health problems associated with Bridgeport Oil and Rental contamination of the Delaware River, Bridgeport, New Jersey
- U.S. Environmental Protection Agency - Evaluation of the environmental impact of dredging of the Hudson River for PCBs, New York
- U.S. Environmental Protection Agency - Evaluation of environmental and public health problems associated with LiPari Landfill in New Jersey
- Velsicol Chemical Corporation - Evaluation of health problems associated with Hardeman County Landfill
- U.S. Environmental Protection Agency - Review of environmental and public health information associated with Denny Farm Site, Verona, Missouri
- U.S. Environmental Protection Agency - Review of environmental and public health information associated with Rose Park Landfill, Salt Lake City, Utah
- U.S. Environmental Protection Agency - Evaluation of environmental and public health problems associated with Taylor Road Landfill, Tampa, Florida
- Dow Chemical Company - Evaluation of health problems associated with a degreasing operation in Tyler, Texas
- United States Environmental Protection Agency - Training course director for Toxicology and Risk Assessment for eight southeastern states. (Alabama, Florida, Georgia, Kentucky, Mississippi, North Carolina, South Carolina, Tennessee)
- Monsanto Chemical Company - Evaluation of adverse health effects associated with PCB contamination of feed grain in Michigan

- American Bar Association - Short course concerning the role of expert testimony in environmental litigations.

- Gates Energy - Evaluation of the toxicity of metal hydrides

- Diversitech/Gen Corp. - Evaluation of mortality among PCB exposed tire and plastic fabricators
- BIC - Evaluation of reproductive workplace hazards
- Breed Industries - Assessment of health risks associated with airbags

GRANT SUPPORT

Effect of Environmental Toxicants on Perinatal Development	ES00782	1970-1982
Effect of Marijuana on Perinatal Development	DA00141	1971-1974
Schleider Foundation Developmental Toxicology		1970-1972
Clinical Pharmacology - Toxicology Center	GM15431	1975-1981
Environmental Toxicology Center	ES00267	1972-1981
Synthesis and Study of New Chelating Agents	ES01018	1975-1981
Life Insurance Research Fund		1970-1981
National Conference on Control of Hazardous Materials Spills	EPA	1977-1978
Wampole Laboratories - Development of a Prenatal Diagnostic Aid for Neurotube Defects		1979-1981
Introduction to Hazardous Materials Incidence Responses and Environmental Hazards Evaluation	EPA	1980-1981
Environmental Toxicant Effects on	ES02824	1981-1986

Perinatal Development		
Study of the Reproductive Toxicity of Selected Chemicals	NF	1985-1987
Studies of Chemical-Induced Toxicity and Stress	ES05216	1988-1994
Studies of Isomer Specific PCB-Induced Toxicity	*Cooperative Agreement	1989-1991
Mechanism of Cocaine-Induced Liver Toxicity	NIDA	1990-1991
Methamphetamine-Induced Toxicity	NIDA	1991-1994
Toxicology Support	**DER/DEP	1991-1995
Radon Risk Assessment	HUD	1994-1996
Pediatric Formula Evaluation	Mead-Johnson	1994-1996
Halocarbon Toxicity	COPH	1995-1996
Assessment of Risk Associated with Vitrified Materials	DOE	1996-1997
Evaluation of Airbag Safety	Breed Industries	1997-1998
Adrenergic Modulation of		
Stress-Induced changes in Pesticide Toxicity	NIOSH-Center for Agricultural Research And Education	1999-2000
Pesticide-Induced Toxicity	NIOSH	1999-2000
PAH-induced Cellular Changes	NIOSH	2000-2001
Environmental Health Monitoring	General Hlth Systems	2000-2003
Florida Biomonitoring	CDC	2001-2003

Arsenic Physician's Workgroup	FL DOH	2001-2002
Adrenergic Alterations of Toxins-Toxicants	DOD	20001-2003
Clinical Toxicology Evaluation of Health Among Residents of Escambia County, FL	CDC	2002-2004
Assessment of Public Health Risks of Blue/Green Algae Cyanobacter	FL DOH	2002-2003
Evaluation of Toxins on DNA	DOD	2003-2004
Brain's DNA Repair Response to Neurotoxicants	DOD	2004-2006

*Department of Environmental and Community Medicine UMDNJ - Robert Wood Johnson Medical School Piscataway, New Jersey
** Dept of Environmental Protection, State of Florida
ES - National Institute of Environmental Health Sciences COPH-College of Public Hlth
DA - National Institute on Drug Abuse DOE- Dept of Energy
GM - National Institute of General Medical Sciences DOD- Dept of Defense
EPA - United States Environmental Protection Agency DOH- Dept of Health
NF - National Foundation March of Dimes
NIDA - National Institute on Drug Abuse HUD - Housing and Urban Development
NIOSH-National Institute of Occupational Safety & Health CDC-Ctr Disease Control

TEACHING EXPERIENCE

Medical Toxicology	Vanderbilt Medical Center Second Year Medical Pharmacology
Developmental Pharmacology	Vanderbilt Medical Center
Drug Metabolism	Two Hour Graduate Level Course Vanderbilt Medical Center
Toxicology	Two Hour Graduate Level Course Vanderbilt Medical Center
Medical Toxicology	Second Year Medical Pharmacology University of Arkansas for Medical Sciences
Advanced Toxicology	Two Hour Graduate Level Course University of Arkansas for Medical Sciences
Oncology	Two Hour Graduate Level Course University of Arkansas for Medical Sciences
Chemical Carcinogenesis	Second Year Medical Pathology University of Florida, College of Medicine

Medical Toxicology	Second Year Medical Pharmacology University of Florida, College of Medicine University of South Florida, College of Medicine
Mechanism of Chemical-Induced Toxicity	Two Hour Graduate Level Course University of Florida, College of Medicine
Introductory Toxicology	Three Hour Graduate Level Course University of Florida, College of Medicine
Health Implications/ Aspects of Chemical Issues	Superfund University Training Institute East Tennessee State University, University of Virginia, Environmental Protection Agency
Risk Assessment • Industrial • Occupational • Environmental	Two Hour Graduate Level Course University of South Florida, College of Public Health
Occupational and Environmental Medicine	Two Hour Graduate Level Course University of South Florida, College of Public Health
Biomonitoring	Two Hour Graduate Level Course University of South Florida, College of Public Health

CONTINUING EDUCATION:

National Hazardous Materials Training Course	Eight Hours (Toxicology)
Hazardous Waste Management	Four Hours (Toxicology)
Toxic Substance Control	Eight Hours (Toxicology)
Environmental Protection Agency, Region IV, Health and Safety Training School	Six Hours (Toxicology)
Industrial Toxicology	Five Hours (Carcinogenesis, Teratogenesis)
Forensic Medicine	Three Hours (Toxicology)

Toxicology and Risk Assessment	Eight Hours
Certification for Hazardous Materials Managers	Twenty Four Hours (NIOSH - ERC)
Neurology and Solvent Encephalopathy	Eight Hours (NIOSH - ERC)
Toxicology and Risk Assessment	Eleven Hours (NIOSH - ERC)

GRADUATE TRAINING - PREDOCTORAL

	Year Degree Conferred	Present Address
Bernardo Mantilla-Plata, Ph.D.	1972	University of Antioqua Department of Toxicology Medellin, Columbia
Michael Stevens, Ph.D.	1973	Monsanto Chemical Company Toxicology Department St. Louis, Missouri
Richard W. Freeman, Ph.D.	1980	Ecology and Environment, Inc. Tallahassee, Florida
Michael E. Fant, M.D., Ph.D	1980	Department of Pediatrics Washington University, School of Medicine St. Louis, Missouri
Adeline Smith, Ph.D.	1982	National Institute of Health Div. of Molecular Toxicology Bethesda, Maryland
James Jernigan, Ph.D.	1983	Amoco Chicago, Illinois
Christopher Teaf, Ph.D.	1985	Florida State University Center for Biomedical and Hazardous Waste Research Tallahassee, Florida
Felix Adatsi, Ph.D.	1986	Department of Natural Resources State of Michigan Lansing, Michigan

M. Ann Clevenger, Ph.D.	1987	EPA Washington, D.C.
Glenn C. Millner, Ph.D.	1987	Environmental Consultant Little Rock, Arkansas
Henry F. Simmons, M.D., Ph.D.	1988	Division of Clinical Toxicology University of Arkansas for Medical Little Rock, Arkansas
Mary Alice Smith, Ph.D.	1989	University of Georgia Athens, Georgia
Hudson K. Bates, Ph.D.	1989	Research Triangle Institute Research Triangle Park, NC
Roland Garipay, MSPH	1998	United States Navy Norfolk, Virginia
Todd Stedeford,Ph.D.	2000	Institute of Molecular Toxicology Kyoto, Japan
Deborah Price	2001	Environmental Health Pinellas County, FL
Rony Francois	2003	Public Health Practice University of South Florida College of Public Health

GRADUATE TRAINING - POSTDOCTORAL

	Years of Study	Present Address
Michael Evans, Ph.D.	1973-1976	Amer. Inst. Toxicology Indianapolis, Indiana
Chandrahar Dwivedi, Ph.D.	1973-1976	Meharry Medical College Dept. of Pediatrics Nashville, Tennessee
Richard P. Koshakji, Ph.D.	1973-1976	Dept. of Pharmacology Vanderbilt Medical Center Nashville, Tennessee

James S. MacDonald	1975-1977	Merck Institute for Therapeutic Research West Point, Pennsylvania
Daniel Goodman, Ph.D.	1978-1980	Univ. of Arkansas Med. Sci. Div. of Interdis. Toxicology Little Rock, Arkansas
Robert C. James, Ph.D.	1979-1981	TERRA, Inc. Tallahassee, FL
Peter Wells, Pharm.D.	1979-1981	University of Toronto College of Pharmacy Toronto, Ontario, Canada
Shahata El-Sewedy, Ph.D.	1982-1983	University of Alexandria Medical Research Institute Alexandria, Egypt
Syed F. Ali, Ph.D.	1982-1983	Natl. Ctr. for Toxicol. Res. Jefferson, Arkansas
Jay Gandy, Ph.D.	1986-1987	Univ. of Arkansas Med. Sci. Dept. of Pharm. & Tox. Little Rock, Arkansas
Christopher Borgert	1991-1992	Univ. of Florida Dept. of Pathology Gainesville, Florida
Robert Demott	1993-1994	Environ Tampa, Florida

PUBLICATIONS

1. Harbison, R.D., R.C. Boerth and J.L. Spratt. Quantitative determination of free and conjugated bilirubin by diazo coupling and a liquid-extraction and column-chromatographic technique. Biochem. J. 104:46c-47c, 1967.

2. Harbison. R.D. and J.L. Spratt. Novobiocin-induced hyperbilirubinemia and its reduction by phenobarbital pretreatment. Toxicol. Appl. Pharmacol. 11:257-263, 1967.

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Toxicant Profile - Methylene Chloride
Prepared for Florida Department of Environmental Regulation

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and blood tests, urine tests.

Q. And how do you determine exposure history?

A. The exposure history is basically taking a good exposure history as we are trained in occupational-environmental medicine.

It's a big part of our training and it expands from the general medicine training where you take a diagnostic oriented history and the occupational history.

You ask about circumstances of exposure, conditions, modifying effects, temporary effects, location, information. You ask questions about exposure, nature, is it chemical, biological, physical.

You look at the duration of exposure but social aspects of that and you look at the connection between exposure and disease presentation and consider, you know, the general available science and knowledge on that subject to make a connection.

Q. Have any studies established a connection between exposure to stachybotrys and adverse human health reactions?

EXHIBIT 3

The second time was 11/5/2004.

Q. Okay. You, in respect to the March 27, 2003 examination, you filled out, it looks like, I'd call it a diagnosis sheet.

What would you call that?

A. Yes. Billing sheet.

Q. I'm sorry?

A. A billing sheet that lists diagnosis.

Q. What did you indicate your diagnosis or impressions were with respect to the visit of March 27 on that billing sheet or diagnosis sheet?

A. I checked off restrictive lung disease, extrinsic alveolitis hypersensitivity, pnemonitis, organic dust exposure, sinusitis chronic, rhinitis, chronic, allergy unspecified.

Q. Okay. What does that mean, "Allergy unspecified"?

A. It means allergy. There are all kinds of allergies so I think that's how the coding goes, ICD coding. So it's allergy essentially.

Q. And what is extrinsic allergic alveolitis HP?

A. It's an inflammatory condition that

affects the lung and can show upper interstitial abnormalities shown on x-rays.

In other words, it's HP, hypersensitivity pneumonitis. Another word would be "farmer's lung" which she's not a farmer so it's not appropriate.

Q. Did you see that based on x-rays?

A. Well, that was my preliminary impression at the time. That was my suspected diagnosis.

There were x-ray findings that did support, would support the diagnosis as such. The history was supportive as such.

Q. Okay. What is restrictive lung disease?

A. It's a disease of hypersensitivity pneumonitis or extrinsic allergic alveolitis can present. This a restrictive lung disease where basically you're not able to take a deep breath and presents with shortness of breath and exertion dyspnea.

Q. What is chronic sinusitis?

A. It's a condition that affects your sinuses, an inflammatory condition that can

A. Well, I indicated symptoms that were still present and significant for her like dyspnea, cough, elevated immunoglobulins which we just talked about and allergy.

Q. And with respect to the ones that were checked in March of 2003, you did not find those in November of 2004, the ones that were checked in March and not checked in November?

A. Well, based on the clinical exam, there wasn't any history, you know, following that for these things.

Q. So, for example --

A. But I have to say the hypersensitivity pneumonitis result of a clearing of the x-ray I had not seen when I filled this out, so I left it out because -- actually, I don't really know why I left it out, but it may as well be there.

Q. You also didn't check chronic sinusitis and chronic rhinitis?

A. Right.

Q. And restrictive lung disease?

A. Yes.

Q. Okay. All right. Now, you said a

moment ago that you have a working diagnosis. What is your working diagnosis?

A. Which is she has an inflammatory lung condition that has not resolved.

Q. And what was the cause of the inflammatory lung condition in your opinion?

A. Well, based on the history and findings, with a reasonable degree of medical certainty, I think it was a microbial exposure in her apartment.

Q. When you say "microbial exposure," what microbes?

A. The fungi and bacteria in her apartment.

Q. What fungi and bacteria?

A. I can't give you specifics and the testing is not sufficient to give you the whole spectrum of what is there.

Certainly the test results that were done by the health department are indicative of what we consider atypical molds in a brick building.

Specifically the things that were identified at the time where a high presence of

A. Yes. I have considered that. I don't think it is.

Q. Why don't you think it is?

A. Because I don't think she has pneumonitis, there's no history of aspiration pneumonia, that type of thing. I can't see it at this point.

Q. Do you believe it has contributed to her problems?

A. I think it may have contributed to some of the other symptomatology she was describing and there may be something to that and that's why I'm not sure I would relate it to the fungal exposure.

I don't think for the pulmonary effects and the blood test results and the breathing test and the chest x-ray results, that it's of any significance.

Q. Is your opinion that the pulmonary inflammation and the related symptoms was caused by an allergic reaction or by a toxic reaction?

A. Well, my thinking is it's probably somewhat a combination of both but primarily an allergic mechanism, something that's best

described now as a type 3.

You know, if you categorize it, type 3, type 4 response in the Gal and Coombs categories, you have a systemic effect where the Immunoglobulin G basically get activated and show up in testing. I don't think it's a medial type allergy and it's not a primary toxic effect.

I don't think, per se, that, you know, stachybotrys or whatever was the primary cause of her problem.

Q. Do you -- is it your opinion that there is support in the scientific and medical literature for exposure to the condition she was exposed to causing the pulmonary inflammation that she suffered?

A. Oh, there's a quite a bit. Jordan Fink has written about it. He has seen patients with that, in Scandinavia. People have described that. Clearly, it's a rare condition.

I don't think most people will respond in these circumstances the way she did and maybe there was something else there that we didn't identify that made her respond this way.

Like her partner, for instance.

Mold-Induced Hypersensitivity Pneumonitis

Paul A. Greenberger, M.D.

ABSTRACT

Mold-induced hypersensitivity pneumonitis results from macrophage- and lymphocyte-driven inflammation, which may be attributable to contaminated humidifiers or heating-ventilation systems or sources in homes, schools, or workplaces. A case may be suspected when there is water intrusion or inadequate drainage. Some fungal causes include species of Alternaria, Aspergillus, Cryptostroma, Penicillium, Pullularia, Rhodotorula, *and* Trichosporon. *The differential diagnosis includes mold-induced asthma, sick building syndrome, mass psychogenic illness (epidemic hysteria), unjustified fears of "toxic" molds, and conditions causing recurrent pneumonitis.* (Allergy and Asthma Proc 25:219–223, 2004)

Mold- or fungal-induced hypersensitivity pneumonitis results in pulmonary infiltrates, restrictive pulmonary function findings, and precipitin reactions to the incriminated fungus. Acute exposures may cause fever and dyspnea 5–20 hours after exposure, obscuring a temporal association. An environmental history for work or home is important, and some issues to consider include those of the home and work environments (Tables I and II). The work environment may be blamed for respiratory complaints, some of which possibly could be attributable to molds. In considering the work environment, some additional considerations are presented in Table II.

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CLASSIFICATION OF HYPERSENSITIVITY PNEUMONITIS

There are four stages of hypersensitivity pneumonitis, although, classically, three stages have been used (Table III). The acute stage classically occurs 4–12 hours after exposure[1] but has been reported to begin as long as 22 hours after exposure to a mushroom spore extract.[2] The temporal relation to the exposure may or may not be appreciated by the patient. If a chest roentgenogram is obtained, there will be nodular or interstitial infiltrates. Pulmonary function tests obtained after bronchoprovocation challenges show restrictive findings including reductions in the forced vital capacity (FVC) and forced expiratory volume in 1 second (FEV_1), with a preservation or increase in FEV_1/FVC consistent with restriction. The diffusing capacity for carbon monoxide will be reduced. During bronchoprovocation with a mushroom spore extract (*Pholiota nameko*), the diffusing capacity decreased from 90 to 55% of predicted while the vital capacity only dropped from 90 to 86%.[2] At the same time, the patient's temperature increased from 36.7 to 39.1°C and the white blood count increased from 5100 to 16,000/μL. Symptoms included dyspnea, cough, and fatigue, which resolved over a period of hours.[2] It is possible to develop arterial hypoxemia or even acute respiratory failure. Avoidance of the antigen is advisable!

The subacute stage consists of dyspnea, fatigue, and nonproductive coughing associated with fever and arterial hypoxemia that occurs intermittently over weeks or months. The chest roentgenogram may be negative when the patient is examined in-between episodes but a high-resolution computed tomography (CT) examination of the lung will show a ground-glass pattern. Some patients will have nodular or linear infiltrates depending on the proximity to an exposure. The subacute clinical symptoms will appear acute if the patient is examined hours after exposure. Avoidance of the antigenic source of course is critical but may be overlooked if the diagnosis has not been made. Pulmonary function parameters can appear either restrictive or normal. In contrast to most forms of hypersensitivity pneumonitis in the

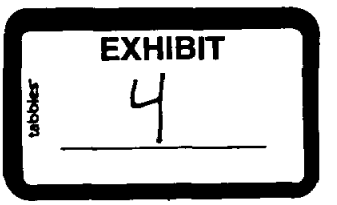

TABLE I

Evidence for Molds in the Home or Work Environment

Evidence of past water intrusions on basement walls
Discolored ceiling tiles from roof leaks
Loose floor tiles
Plumbing fixtures with leaks
Musty odors
Discolored or very clean basement carpeting
Moldy vents in the air conditioning or heating systems
Moldy bathroom tiles and walls

TABLE II

General Issues to Consider in the Work Environment

A likely occupational etiology, e.g., mushroom cultivation, malt production, etc.
Is the air filtered effectively and the system maintained on schedule?
Other VOCs present?
Is the air conditioning condenser turned off at night?
Are roof leaks causing mold growth?
Is there overcrowding of employees?
Is there inadequate ventilatory capacity with increased concentrations of CO_2?
Is the location of the air intake too close to roads or idling trucks?
Is there short circuiting between exhaust and supply vents?

VOCs = volatile organic compounds.

subacute stage, avian hypersensitivity pneumonitis may result in obstructive or restrictive pulmonary function changes.[3,4] Patients with subacute hypersensitivity pneumonitis should have a favorable outlook if the antigenic exposure is avoided.

The designation subacute chronic hypersensitivity pneumonitis was proposed to describe a patient who had repeated episodes of subacute hypersensitivity pneumonitis that resulted from a contaminated humidifier in her bedroom.[5] The patient had precipitin reactions to 10 fungi and humidifier water.[5] Her CT scan showed a diffuse ground-glass pattern with thickened alveolar septa. A lung biopsy revealed patchy areas of lymphocytes, plasma cells, and histiocytes "in a bronchiolocentric pattern."[5] Her diffusing capacity for CO was 57% of predicted, and the FVC was 95%. The FEV_1 was 91%. The humidifier was removed and she received a 5-week course of prednisone. Her diffusing capacity remained unchanged and the FVC had declined from 2.84 L (95%) to 2.48 L (85%). Over almost 4 years, although the patient had not experienced dyspnea as she had at the time of diagnosis, she did have a nonproductive cough. On examination, there were fine, bibasilar crackles. The diffusing capacity was at 61% and the FVC was at 2.51 L(86%).[5] She had only two positive precipitin reactions that were to *Aspergillus flavus* and *Penicillium herbarum*. Unexpectedly, the chest CT was worse with pulmonary fibrosis, interlobular septal thickening, honeycomb formation of the left lower lobe, and areas of ground-glass fibrosis. Her pulse oximetry revealed a baseline of 98% and a drop to 92% with exercise.[5] The initial improvement in this patient was associated with radiographic progression on chest CT examination and lack of improvement of pulmonary function parameters. The lack of improvement of the diffusing capacity and other pulmonary function tests and appearance of honeycomb fibrosis on CT suggest a course in-between subacute and chronic hypersensitivity pneumonitis.[5]

TABLE III

Classification of Hypersensitivity Pneumonitis

Acute
Subacute
Subacute chronic
Chronic

Chronic hypersensitivity pneumonitis consists of irreversible restrictive pulmonary function tests, chronic fibrotic radiological findings, lack of or a partial response to oral corticosteroids, and the potential for need for supplementary oxygen. The chest examination will show crackles, perhaps in many areas not just the bases. Some patients will have weight loss and irreversible dyspnea can be expected. It is still possible to have subacute episodes if exposure to the antigen occurs. Rare fatalities from chronic hypersensitivity pneumonitis have been reported from fungi,[6] other cases being from either avian antigens[7,8] or thermophilic organisms (classified as bacteria) from farmer's lung.[9,10]

Identification of hypersensitivity pneumonitis in the acute or subacute stages is the goal so that avoidance of the antigens can occur and respiratory status can be preserved as best as possible. In Table IV, some suggestions for making the diagnosis of mold (fungal)-induced hypersensitivity pneumonitis are listed.

IMMUNOLOGIC FINDINGS IN HYPERSENSITIVITY PNEUMONITIS

Precipitating antibodies are necessary but not sufficient because they represent immunoglobulin G antibodies, which can be evident in exposed subjects as well. Sera should be concentrated and reactive antigens are crucial. Certain cases of hypersensitivity pneumonitis are overlooked because of poorly reactive antigens being used in the precipitin reaction testing. Bronchoalveolar lavage reveals a clear reversal of the usual CD4/CD8 2:1 ratio.[1] Increases in the $CD8^+$ lymphocyte population to as much as 60–80% in both symptomatic and asymptomatic subjects with hypersensitivity pneumonitis can occur. In contrast, control (non-hypersensitivity pneumonitis) bronchoalveolar lavage may have 13% lymphocytes because the predominant cell in

TABLE IV

Suggestions for Making the Diagnosis of Mold-Induced Hypersensitivity Pneumonitis

History of mold exposure and potential sources of exposure
Symptoms of cough, dyspnea, fever, and, in some patients, myalgias
Chest roentgenogram or CT examination (nodular infiltrates, ground-glass opacification, or fibrosis)
Precipitins in gel to mold (fungal) antigens
Restrictive pulmonary function tests or at least decreased diffusing capacity for CO
Lung biopsy specimen (not always necessary) showing noncaseasting granulomas consistent with hypersensitivity pneumonitis and absence of other conditions

lavage is macrophages. The lymphocytes are activated as shown by the presence of the receptor for interleukin (IL)-2 ($CD25^+$), which is shed from activated IL-2.[1] IL-2 increases could help explain the lymphocyte predominance in bronchoalveolar lavage. The $CD8^+$ lymphocytes are activated but their suppressor activity is limited; perhaps this effect allows for the antigens being proinflammatory instead of being contained. Various pivotal cytokines such as tumor necrosis factor α, interferon γ, and IL-1β trigger increases in a key receptor soluble intracellular adhesion molecule (sICAM). Furthermore, tumor necrosis factor α contributes to granuloma formation (and containment of *Mycobacteria*[11]). Interferon γ increases macrophage antigen presentation, stimulates other cells such as natural killer ($CD56^+$) cells, and its presence is consistent with $CD4^+$ T helper cell subset 1 activation. Cellular influx, including polymorphonuclear leukocytes, into tissue occurs through ICAM. The bronchoalveolar lavage lymphocytes and macrophages express increased levels of ICAM-1 compared with controls.

Lymphocyte suppression is impaired in some cases of hypersensitivity pneumonitis.[1] *In vitro* mitogen responses of the bronchoalveolar lavage lymphocytes are reduced when phytohemagglutinin or concanavalin A are used, consistent with reduced suppressor activity. When additional alveolar macrophages, serving as accessory cells, are incubated with separated $CD4^+$ or $CD8^+$ lymphocytes, the responses to mitogens return to normal. Thus, although there is a remarkable bronchoalveolar lavage lymphocytosis, lymphocyte responsiveness is impaired. However, it is not irreversible because providing additional macrophages overcomes, at least *in vitro*, the lymphocyte suppression.

Costimulatory molecules on macrophages in bronchoalveolar lavage are increased.[12] When bronchoalveolar lavage cells are stained with anti-CD80 (B 7–1) and anti-CD86 (B 7–2), in hypersensitivity pneumonitis, anti-CD80 was positive in 35% of macrophages and anti-CD86 was evident in 62% of macrophages. In contrast, control macrophages were positive with anti-CD80 in 7% and anti-CD86 in 24%. These findings suggest that the macrophages are activated, having phagocytosed the specific antigens. The macrophages appear to be more effective at antigen presentation to T lymphocytes because of their increased extent of activation. There were no differences found between the T lymphocytes staining positive for CD28 (receptor for CD80) or cytotoxic T lymphocyte antigen (CTLA)-4 (receptor for CD86). These studies are consistent with increased accessory molecule expression, which would support greater antigen presentation to T lymphocytes. In addition, when analysis of bronchoalveolar lavage lymphocytes was studied, there was a reduction in apoptotic lymphocytes compared with normals.[13] The immunologic responses in terms of suppression are impaired so that lymphocyte activation[12–14] is not contained. Granulomas are noncaseating and are considered to occur because of chronic antigen stimulation.[11] Alveolar macrophages have been reported to generate increased reactive oxygen species such as superoxide anion.[15] Indeed, the contribution of superoxide anion, often thought attributable to polymorphonuclear leukocytes, results from activated alveolar macrophages. The complexity of hypersensitivity pneumonitis can not be underestimated; this condition is an unusual lung disease because of the predominance of $CD8^+$ lymphocytes in bronchoalveolar lavage, exaggerated macrophage and lymphocyte activation, multiple causes, and variable prognosis.

CAUSES OF MOLD-INDUCED HYPERSENSITIVITY PNEUMONITIS

Some of the many examples of mold-induced hypersensitivity pneumonitis are listed in Table V. The causes may be remedied to reduce the incidence of newly affected workers. Nevertheless, the nature of the manufacturing or recovering process still may allow for workers to become sensitized. For example, there are many uses of cork obtained from tree or ground bark that becomes moldy causing suberosis.[16] *Penicillium frequentans* and *Aspergillus fumigatus* also caused the disease. In malt worker's lung, barley must be steeped in water so that it converts to malt. The process may occur on a wet floor 20 × 120 ft. This step in the process allows for the possibility of mold growth and disease production.

A PARTIAL DIFFERENTIAL DIAGNOSIS REGARDING MOLD-INDUCED HYPERSENSITIVITY PNEUMONITIS

Table V lists true causes of mold- or fungus-related hypersensitivity pneumonitis. Some other conditions that should be considered and excluded are presented in Table VI. Significant diagnostic expertise may be needed to rule in mold-induced hypersensitivity pneumonitis and exclude some of these syndromes. In sick building syndrome, there is an excess of work-related symptoms reported.[17]

TABLE V

Causes of Mold-Induced Hypersensitivity Pneumonitis

Name	Fungal Cause or Circumstance
Summer type	Moldy rugs stacked on each other, *Trichosporon cutaneum*
Humidifier disease	*Penicillium* species, *T. vulgaris, S. virdis*
Avian	*Aspergillus fumigatus* in bird excreta
Malt worker's lung	*Aspergillus clavatus, A. fumigatus*
Suberosis	Moldy cork dust, *Penicillium* species, *A. fumigatus*
Wood worker's lung	*Alternaria* species
Sequoiosis	Moldy wood dust, *Pullalaria* species, *Penicillium expansum* on a moldy floor
Cheese worker's lung	*Penicillium roqueforti, P. caseii*
Sauna taker's lung	*Pullalaria* species on moldy cedar
Maple bark stripper's disease	*Cryptostroma corticale*
Soy sauce brewer's disease	*Aspergillus oryzae*
Ventilator lung or humidifier disease	Some fungi or thermophillic bacteria
Moldy basement walls	*Aspergillus* species, *Rhodotorula* species

TABLE VI

Conditions That May Be Confused with Mold-Induced Hypersensitivity Pneumonitis

Mold (fungal)-induced asthma
Building-related syndromes (true disease)
Asthma
Hypersensitivity pneumonitis from another cause
Legionella pneumonia
Sick building syndrome
Mass psychogenic illness
Unjustified fears of "toxic" molds

Symptoms may be cutaneous, mucous membrane irritation, fatigue, headache, or difficulty concentrating. Some episodes are explained by certain conditions affecting heating-cooling systems or even introduction of vehicle exhaust fumes into the intake air of the building. Atopy is not a risk factor for reporting symptoms. In one study, doubling of the airflow in the building did not reduce the number of reported symptoms. Another condition is mass psychogenic illness.[18] This condition is epidemic hysteria in which a constellation of symptoms suggestive of an organic illness is reported but there is no identifiable cause found. The affected subjects, who are healthy before the onset of this condition, share beliefs as to what is the cause often related to detection of a noxious odor such as a gasoline smell. One person is affected by the odor and then many more are as well. The condition is an example of verbal person-to-person transmission. There may be sudden onset of headaches, shortness of breath, and dizziness, but despite intensive efforts, no causative agent is identified.[18] In one example triggered by the recognition of a smell of gasoline, volatile organic compounds, pesticides, carbon monoxide, mercury, paraquat, and polychlorinated biphenyls all were excluded.[18]

SUMMARY

Hypersensitivity pneumonitis from molds may be suspected and then confirmed by a constellation of findings such as precipitin reactions, a probable source, lung CT findings, restrictive pulmonary function parameters, and, in some cases, the lung biopsy. Remediation of the water source promoting fungal growth is advisable, but some patients will have residual pulmonary fibrosis. The differential diagnosis also includes mass psychogenic illness, unjustified fears from "toxic" fungi, true building-related conditions such as mold-induced asthma, and sick building syndrome. Because fungi can be cultured from indoor environments, they may be blamed for more illness than justified. Nevertheless, when the clinical and radiological features suggest mold-induced hypersensitivity pneumonitis, serum precipitins should be obtained and a short course of prednisone often is administered. Attempts at remediation of the suspect environment should be advised.

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17. Hypersensitivity Pneumonitis

Rachel E. Story, M.D., M.P.H., and Leslie C. Grammer, M.D.

Hypersensitivity pneumonitis (HP), also referred to as extrinsic allergic alveolitis, is characterized by non-immunoglobulin E (IgE)-mediated inflammation of the parenchyma, alveoli, and terminal airways of the lung initiated by inhaled antigens in a susceptible host. Historically, HP was associated primarily with occupational exposures such as thermophilic actinomycetes on hay resulting in farmer's lung. Residential contact with agents responsible for HP now accounts for most new cases of the disease with pet birds, contaminated humidifiers, and indoor molds being the most frequent sources of exposure.[1]

The mechanism of inflammation in HP is not elucidated fully. The inflammation is non-IgE mediated and seems to be driven by activated macrophages and $CD8^+$ cytotoxic lymphocytes that are unable to suppress macrophage activity.[2] Elevated antibody production, primarily IgG, is seen in HP and is thought to be secondary to $CD4^+$ T-helper lymphocyte stimulation of plasma cells.[1]

Etiologic agents of HP are either organic high molecular weight compounds such as bacteria, fungi, amoebae, and animal proteins or inorganic low molecular weight haptens such as isocyanate and drugs including amiodarone, gold, and minocycline. A comprehensive list of etiologic agents is beyond the scope of this article but can be found elsewhere.[1,2] Examples include: thermophilic actinomyces found in moldy hay causing farmer's lung, *Naegleria gruberi* in contaminated humidifiers causing ventilation pneumonitis, avian protein in bird droppings causing bird-fancier's lung, and acid anhydrides in plastics causing plastic worker's lung.

The epidemiology of HP varies with the etiologic agent, population, and environment. Although thermophilic actinomyces and avian proteins cause most cases of HP in the United States, the incidence varies by region and season with farmer's lung more common in humid regions and seasons. In Japan, summertime mold contamination of homes with *Trichosporon cutaneum* and *Cryptococcus albidus* is the most common cause of HP.

HP presents in an acute, subacute, and chronic form depending on the amount and duration of exposure and the level of host reactivity. The acute form typically occurs within 6–12 hours of an intense exposure with symptoms of fever, dyspnea, and nonproductive cough. The chronic form is associated with continuous low-level exposure and presents with insidious onset of shortness of breath, productive cough, and weight loss. The subacute form has features of both the acute and the chronic forms. Table I has additional information distinguishing the three forms of HP.

A high index of suspicion is required to make a diagnosis of HP. There is no single diagnostic test that is definitive for HP. Physicians must identify exposure to an agent capable of causing HP with an appropriate temporal relation to symptoms. Diagnostic tests are used to support the diagnosis. For the acute form of HP, an inhalation challenge can clearly establish exposure and an appropriate temporal relation to symptoms, but it is rarely done because severe respiratory reactions may occur.

Skin testing is not useful because HP is not an IgE-mediated disease. High levels of serum precipitating IgG antibodies to the offending antigen are found in HP but are not diagnostic because many individuals with exposure but no disease have high levels of precipitating antibodies. The results of physical examination, chest x ray, and pulmonary function test differ depending on the form of HP. Details of the differences are shown in Table I. Lung biopsy is not diagnostic but often is characterized by poorly formed granulomas, alveolar wall infiltration with lymphocytes, plasma cells and neutrophils, and, in the chronic form of HP, fibrosis.[2]

The cornerstone of management in HP is early diagnosis and avoidance of the offending agent. Avoidance often requires a change in occupation or parting with a beloved pet. Therefore, frequently, emotional, psychological, social, and economic factors lead to noncompliance. Prednisone at a dose of 0.5 mg/kg per day can decrease symptoms in the acute and subacute forms but has no benefit in terms of disease progression. It is dangerous to treat a patient with steroids in the setting of continued antigen exposure.

The majority of patients with HP enjoy a complete recovery once the offending agent is identified and avoided. However, deaths have been reported if exposure continues.[2]

IMMUNOLOGY

- The inflammation in HP is not IgE mediated.
- Activated macrophages and $CD8^+$ cytotoxic lymphocytes are key mediators of inflammation in HP.

TABLE I

Clinical Presentations of HP

Feature	Acute	Subacute	Chronic
Fever, chills	+	−	−
Dyspnea	+	+	+
Cough	Nonproductive	Productive	Productive
Malaise, myalgia	+	+	+
Weight loss	−	−	+
Rales	−	+	+
Chest film	Nodular Infiltrates	Nodular Infiltrates	Fibrosis
PFTs	Restrictive	Mixed	Mixed
DLCO	Decreased	Decreased	Decreased

PFTS = pulmonary function test; DLCO = diffusing capacity for carbon monoxide.
Source: Ref. 2 (with permission).

- Bronchoalveolar lavage fluid typically reveals a lymphocytosis with a ratio of $CD8^+$ cells to $CD4^+$ cells greater than one.
- Activated macrophages have increased expression of CD80/CD86 and T cells have increased expression of its counter-ligand CD28.
- Increased expression of CD80/CD86 and CD28 increase the inflammation seen in HP.

CLINICAL PEARLS

- HP is a clinical diagnosis that requires a high index of suspicion to detect.
- Residential exposures to causative agents are responsible for most new cases of HP.
- Clinical features and diagnostic test results differ in the acute, subacute, and chronic forms of HP
- Skin testing is not useful because HP is not an IgE-mediated disease.
- The cornerstone of management in HP is early diagnosis and avoidance of the offending agent.
- Prednisone will decrease symptoms in the acute and subacute phases but will not impact disease progression in most cases.

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Toxic and Other Non–IgE-mediated Effects of Fungal Exposures

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There are more than 100,000 recognized species of fungi, comprising 25% of the biomass of the earth. Allergic, IgE-induced, manifestations of airborne fungi are common, whereas non-IgE manifestations are rare. Recently, much focus has been placed on the non–IgE-mediated effects of various molds, including hypersensitivity pneumonitis, infectious disease, and mycotoxicoses. Hypersensitivity pneumonitis is a clinical syndrome associated with systemic and interstitial lung disease that occurs in susceptible individuals following fungal inhalation. Most fungi are not pathogenic to man; however, certain fungi are capable of infecting immunocompetent individuals. Although mycotoxins and exposure to mycotoxins ("toxic mold syndrome") are implicated in causing numerous, nonspecific, systemic symptoms, currently, there is no scientific evidence to support the allegation that human health is affected by inhaled mycotoxins. However, if mold is discovered in a home, school, or office setting, the source should be investigated and appropriate remediation undertaken to minimize structural damage and potential allergic sensitization.

Introduction

Fungus, commonly referred to as mold, is used to describe the different members of the Fungi kingdom, of which there are five main phyla including Chytridiomycota, Zygomycota, Ascomycota, Basidiomycota, and Deuteromycota. Fungi are eukaryotic, unicellular, dimorphic, or filamentous and are usually spore-bearing organisms. There are more than 100,000 recognized species of fungi, comprising 25% of the biomass of the earth, occurring in numerous forms, such as mushrooms, toadstools, puffballs, yeasts, mildew, smuts, molds, and rusts. Fungi can cause human disease via four mechanisms, including allergy, infection, non–IgE-mediated hypersensitivity, and toxicity. Allergic, IgE-induced, manifestations of airborne fungi include asthma, conjunctivitis, rhinitis, allergic sinusitis, and allergic bronchopulmonary aspergillosis. Recently, much focus has been placed on the non–IgE-mediated effects of various molds. The major non-IgE immunologic disease of molds is hypersensitivity pneumonitis. This paper is focused on the non-IgE effects of mold in humans, including hypersensitivity pneumonitis, infectious disease, and mycotoxicoses (including the recently described "toxic mold syndrome").

Hypersensitivity Pneumonitis

Fungal antigens inhaled as biologic dusts can induce pulmonary disease through non-IgE immune mechanisms in immunocompetent patients. The terms hypersensitivity pneumonitis (HP) and extrinsic allergic alveolitis are used to describe this syndrome. Despite the term alveolitis, there is involvement of the interstitium, alveoli, middle airways, and terminal airways. The syndrome is due to immune mechanisms related to alveolar macrophages, and Th1 lymphocytes are activated by inhalation of antigens by susceptible individuals. The clinical presentation varies depending on the duration and extent of exposure to the fungi and the immunologic response of the individual.

Lung disease in grain workers was first reported by Ramazzini in 1713, and farmer's lung was initially described in England in 1932. In the past 35 years, microorganisms (actinomycetes, bacteria, fungi, amoebae), animals (primarily avian), low molecular weight chemicals, and drugs have been identified as triggers of hypersensitivity pneumonitis.

Epidemiology

There is little information regarding the exposure levels necessary to induce HP in susceptible individuals. The prevalence varies by antigen and by location. Paradoxically, this disease occurs less frequently in smokers than in nonsmokers. Cigarette smoking seems to be protective by affecting alveolar macrophage function, thereby inhibiting lung inflammation when immunogenic particles are inhaled. Another factor important in the induction of disease appears to be the concomitant occurrence of a pulmonary insult, such as a viral respiratory infection [1].

Fungal antigens of hypersensitivity pneumonitis

Fungal antigens have become the most commonly reported causes of HP recently and are summarized in Table 1. Many fungus-related HP syndromes have colorful and descriptive names. Sensitization by fungal antigens occurs through the aerosol route, whereby particles smaller than 5 microns can

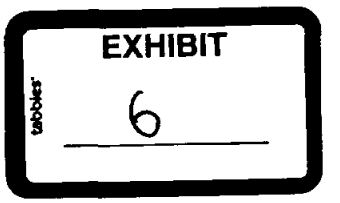

Table 1. Fungal antigens, sources, and diseases for hypersensitivity pneumonitis

Fungal antigen	Source	Disease
Aspergillus species (*fumigatus, clavatus*)	Moldy brewer's malt dust	Malt workers lung
	Moldy esparto grass in stucco	Stipatosis
	Compost	Compost lung
	Moldy tobacco	Tobacco workers disease
	Contaminated O_2 humidifier	
	Contaminated soy sauce	
Alternaria species	Moldy wood dust	Wood workers lung
Rhizopus and *Mucor* species	Moldy wood trimmings	Wood trimmers disease
Botrytis cinerea	Moldy grapes	Wine grower's lung
Aureobasidium pullulans	Contaminated HVAC system	Air-conditioner lung
Cladosporium species	Contaminated sauna	Sauna taker's lung
Cephalosporium species	Sewer-water–contaminated basement	Cephalosporium HP
Penicillium frequentans	Moldy cork dust	Suberosis
Penicillium caseii and *roqueforti*	Cheese mold	Cheese workers lung
Penicillium brevicompactum, Fusarium, Absidia corymbifera, Wallemia sebi	Moldy hay or cowshed fodder	Farmer's lung
Penicillium (*expansum, cyclopium, chrysogenum*)	Moldy wood dust	N/A
Penicillium (*camembertii, nalgiovense, chrysogenum*)	Salami seasoning	Salami workers lung
Penicillium and *Monocillium* species	Moldy peat moss	Peat moss processors lung
	Moldy oak and maple trees	Woodman's disease
Pleurotus osteatus, Hypsizigus marmoreus, basidiospores	Commercial indoor mushroom cultivation	Mushroom workers lung
Trichosporum cutaneum, T. ovoides, Cryptococcus albidus	Contaminated Japanese house dust	Summer-type HP
Cryptostroma corticale	Moldy maple bark	Maple bark stripper disease
Rhodotorula rubra	Moldy cellar/bathroom walls	
Graphium species, *Alternaria, Aureobasidium pullulans*	Moldy redwood dust	Sequoiosis
Pezizia domiciliana	Moldy home	El Niño lung
Lycoperdon puffballs	Puff ball spores	Lycoperdonosis
Candida species	Moldy reed	Saxophonists lung
Epicoccum nigrum	Moldy basement shower	Basement shower HP
Fusarium napiforme	Moldy home	N/A
Saccharomonospora viridis	Dried grasses and leaves	Thatched-roof disease
Streptomyces albus	Contaminated fertilizer	Streptomyces albus HP

HP—hypersensitivity pneumonitis; HVAC—heat ventilation and air conditioning.

enter the alveoli. Organic dusts that contain molds can also contain toxins, including mycotoxins with enzymatic activity that function as adjuvants.

Reports of acute mold-related HP include children exposed to an unventilated shower contaminated with *Epicoccum nigrum* [2], a housewife exposed to *Penicillium expansum* contaminated wood dust [3], the inhalation of basidiospores during processing and packing of mushrooms [4], and an adult who developed HP caused by *Pezizia domiciliana* after house flooding from the rains of El Niño [5]. Species of *Aspergillus* have been implicated in a soy sauce brewer, a farmer with a poorly ventilated greenhouse, a hospitalized patient inhaling contaminated humidified oxygen [6], and Spanish stucco makers working with moldy esparto grass fibers [7]. Suberosis describes affected workers in the cork industry who inhale spores of *Penicillium frequentans*. *Penicillium* spores found on aging cheese and salami have caused cheese worker's lung and salami worker's lung, respectively [8]. Occupational HP has been linked to *Penicillium* spores in moldy wood and peat moss [9]. Fungus-contaminated air-conditioning systems have been implicated in outbreaks of HP [10]. Room humidifiers have been causative sources with *Aureobasidium pullulans* [11] and the red yeast *Rhodotorula* [12]. During the summer in southern Japan, house dust contaminated with *Trichosporon cutaneum* or *Cryptococcus albidus* can cause HP [13].

Clinical presentation

Hypersensitivity pneumonitis can present clinically as acute, subacute, or chronic depending on the nature of the inhaled dust, its antigenicity, the intensity and frequency of exposure, and the immunologic response of the host.

The acute form is characterized by both respiratory and systemic symptoms that begin 4 to 6 hours after exposure. A dry, nonproductive cough, dyspnea, high fever, chills, myalgias, and malaise might persist for 18 hours, followed by spontaneous recovery if the person is removed from the source of antigen exposure. The attacks recur each time the individual is exposed, but the severity can vary. With frequent episodes, symptoms of anorexia, weight loss, and

progressive dyspnea might be prominent, resulting in the chronic form. During an acute attack, bibasilar inspiratory rales are prominent and can persist for weeks. Between attacks, the examination is usually normal.

The subacute form includes progressive respiratory symptoms for weeks to months, without the explosive systemic symptoms noted earlier, unless there is an exceptionally high allergen exposure.

The chronic form can be subdivided into that following recurrent acute attacks or the more difficult to recognize insidious form. Prolonged or continuous exposure to small amounts of fungal antigen can result in the insidious form, manifested as progressive dyspnea, dry cough, malaise, weakness, anorexia, and weight loss. Fever is often absent. The irreversible pulmonary damage occurs without acute attacks. Lung auscultation typically reveals fine, dry crackles, but wheezing is not uncommon. Clubbing is unusual.

Laboratory features

In the acute form, leukocytosis up to 30,000 cells per mm^3 with a left shift might be present. Elevations of all immunoglobulin isotypes, except IgE, erythrocyte sedimentation rate, C-reactive protein, rheumatoid factor, and serum lactate dehydrogenase have been reported. Arterial blood gases reveal a respiratory alkalosis with hypoxemia in the acute form. The hypoxemia is accentuated by exercise. The characteristic immunologic finding is serum-precipitating IgG antibodies against the specific offending fungal antigen as measured by gel diffusion techniques or immunoelectrophoresis. Precipitin bands between the patient's sera and antigens can be detected in nearly all ill individuals but also in up to 50% of asymptomatic similarly exposed individuals [14]. Serum precipitins might disappear with time, if exposure ceases.

Radiography

During an acute attack, soft, patchy, coalescing parenchymal infiltrates can be seen bilaterally. Between acute attacks, the chest radiograph might be normal, although, more commonly, there are very fine nodulations and reticulation with coarse bronchovascular markings. End-stage disease appears as diffuse fibrosis with honeycombing. High-resolution computed tomography (HRCT) is not better than plain radiographs in acute disease; however, in chronic disease, HRCT is superior in identifying abnormal findings, such as centrilobular nodules, ground-glass opacities, and emphysematous changes.

Pulmonary function

Pulmonary function abnormalities can vary depending on the form of the disease. A restrictive ventilatory defect is the most common finding in acute disease occurring 4 to 6 hours after exposure. There is a decrease in both forced vital capacity and forced expiratory volume in 1 second (FEV_1). The diffusion capacity frequently decreases during the attack, reflecting alveolar involvement. Blood gases reveal hypoxemia and respiratory alkalosis, particularly with exercise. The abnormalities disappear as the acute symptoms subside. If the lung damage is severe, volume and flow abnormalities might persist during asymptomatic phases. A mixed restrictive and obstructive ventilatory defect with low diffusion capacity can be seen in chronic HP.

Bronchoalveolar lavage and histopathology

Patients with HP have increases in the absolute numbers of both CD4+ and CD8+ T lymphocytes, and a very prominent increase in CD8+ T cells [15]. Bronchoalveolar lavage (BAL) fluid also contains activated lung macrophages [16], increases in mast cells and natural killer cells, and specific IgG and IgA antibody. Cultures are usually negative.

The histopathology depends on the stage of the disease, but generally appears as nonspecific interstitial pneumonitis (NSIP). In acute disease, there is a marked predominance of lymphocytes, activated macrophages, plasma cells, and neutrophils in alveoli and interstitial spaces. With disease progression, the alveoli become obliterated, and the interstitial process becomes fibroblastic. In chronic disease, there is less prominent lymphocytic alveolitis, but interstitial inflammation includes not only mast cells and plasma cells but also noncaseating granulomas and fibrosis. There is minimal epithelial cell necrosis or connective tissue destruction, but peripheral destruction of alveolar walls can result in honeycombing.

Diagnosis

Criteria for diagnosing HP include: 1) compatible symptoms; 2) evidence of exposure to the appropriate antigen by history or by detection of specific antibodies in serum and/or BAL fluid, 3) chest radiograph or HRCT with compatible findings; 4) lung lavage fluid Th1 lymphocytosis (if BAL is performed); 5) histologic changes such as NSIP (if lung biopsy is performed); and 6) reproduction of the symptoms with supporting laboratory and lung function abnormalities after exposure to the suspected environment. Additional findings that are nearly always present but can also be found in other interstitial lung diseases include: 1) bibasilar dry rales; 2) decreased diffusing capacity; and 3) arterial hypoxemia at rest or during exercise [17].

The suspicion for a causative environmental antigen is a temporal relationship between symptoms and the workplace, hobby, and entrance into a building or home. This relationship is more difficult to establish in the chronic form. Clues to identify mold contamination include evidence of broken-down foundations, deterioration of walls, roof damage, a mildewed odor, "powdery dust" exiting air conditioner registers, and water stains on ceilings or walls. A site visit might be helpful to measure indoor air quality compared with outdoors, and microbial contamination.

There is no single clinical or unique test currently available to confirm the diagnosis of HP. Rather, it is a combination of clinical findings, radiographic abnormalities, pulmonary function, and immunologic testing that sup-

Table 2. Common dermatophytosis and respective pathogens

Type of infection	Pathogen	Remarks
Tinea capitis (scalp)	*Trichophyton tonsurans, T. schoenleini (favus), Microsporum audouini, M. canis*	More common in children than adults
Tinea unguium (nails)	*T. rubrum, T. mentagrophytes*	Fungal nail infections are common, occurring in 5:1000 people
Tinea barbae (beard)	*T. rubrum, T. verrucosum*	Occur mainly in adults handling animals or straw contaminated by infected mice
Tinea corporis (body)	*T. rubrum, T. mentagrophytes, M. canis, T. verrucosum, T. concentricum, M. gypseum*	Facial involvement might involve flares following exposure to sunlight
Tinea cruris (groin)	*Epidermophyton floccosum, T. rubrum*	Very contagious spread via contaminated towels and saunas
Tinea pedis (feet)	*E. floccosum, T. rubrum, T. mentagrophytes*	Infection commonly starts with scaling in the third or fourth interdigital space

ports the diagnosis [18]. Lung biopsy and inhalation challenge might be necessary in some cases, either to confirm the disease or to rule out other diagnoses.

When a specific environment is suspected, pulmonary function tests can be performed before and several hours after exposure. Likewise, purposeful exposure in the laboratory to the suspected antigen can also be done. If significant changes occur in lung volumes or diffusing capacity, the environment should be further investigated with fungal cultures and immunologic studies using the patient's serum. Chest radiographs are suggestive in all forms, but HRCT can further delineate chronic disease, determine the site for lung biopsy, and narrow the differential diagnosis. Skin testing has no practical role in the diagnosis. Open lung biopsy should be performed if other studies do not establish the diagnosis. The findings of NSIP should prompt an investigation for HP; however, NSIP can be seen with connective tissue diseases.

Treatment

Early recognition of HP with subsequent avoidance of the offending fungus is the most important factor in reversal of the disease process. Once identified, a variety of measures can be implemented to avoid the offending fungal antigen. The use of air-filtering systems, masks, or alterations in the forced-air heating or cooling systems might be beneficial. Remediation techniques might be necessary to increase home ventilation, maintain humidity at less than 50%, and treat areas of regular water exposure (*ie*, water traps). Removal of contaminated building materials, which serve as reservoirs for fungal contamination, would likely be necessary. Most individuals can expect complete resolution of symptoms and a return to normal pulmonary function days to weeks after the exposure has stopped, if irreversible lung damage has not occurred.

If the disease appears to be progressing, even with avoidance, oral corticosteroids (*eg*, prednisone 40 to 80 mg/day) are the drugs of choice. The clinical responses to steroids are often dramatic. The duration of therapy is based on clinical improvement. In challenge studies, albuterol helped where there was a decrease in the FEV_1. There are insufficient data on using inhaled corticosteroids. Allergen immunotherapy with the offending fungal antigens is not advisable, because the immune complexes formed might induce a vascular inflammatory process.

Infectious Diseases

Most fungi are not pathogenic to man; however, certain fungi are capable of infecting immunocompetent individuals. Typical examples include the dermatophytes, *Blastomyces, Cryptococcus, Histoplasma, Coccidiodes*, and *Candida*. Immunocompromised individuals are not discussed in this review, but are at increased risk for infection by a variety of opportunistic fungi, typically including *Aspergillus, Fusarium, Zygomycete*, and *Candida*. Susceptible individuals are those with severe cellular immune dysfunction, such as those with HIV infection, those undergoing chemotherapy, those on other immunosuppressive drugs, and those with uncontrolled diabetes mellitus.

Dermatophytosis

Dermatophytosis is a superficial infection of keratinized structures such as the hair, skin, and nails by three genera of dermatophytes including *Trichophyton, Epidermophyton*, and *Microsporum*. These genera contain more than 40 species. The various dermatophytoses are commonly classified according to infected body location preceded by "tinea." Table 2 summarizes the common dermatophytoses. The treatment of dermatophytosis involves the topical application of medications such as clotrimazole, miconazole, or ketoconazole. However, infections of the nails and hair respond poorly to topical treatment modalities and usually require prolonged courses with oral antifungal agents such as griseofulvin.

Blastomycosis

Blastomycosis is caused by the dimorphic fungus *Blastomyces dermatitidis*. It occurs as a result of the inhalation of *B. dermatidis conidia* into the lungs. Person-to-person trans-

mission does not occur. *B. dermatidis* spores are found in soil and are endemic in regions of the Ohio and Mississippi River valleys. Most individuals who inhale *B. dermatidis* do not develop symptoms of blastomycosis. The major clinical manifestations of blastomycosis are pulmonary, cutaneous, and disseminated disease. Pulmonary blastomycosis presents with fever, cough, chest pain, and mucus development and can be self-limited. However, 40% to 80% of patients develop extrapulmonary dissemination, most commonly to the skin, bones, and male urogenital tract. Skin lesions can be ulcerative, verrucous, or nodular. Biopsies of the lesions typically reveal both granulomatous and suppurative inflammation. The diagnosis is based on the identification of the organism in tissues (broad-based, single-budding yeast), tissue culture, or chemiluminescent DNA probe. Treatment is intravenous amphotericin B for severe/disseminated infection and oral ketoconazole, itraconazole, or fluconazole for mild-to-moderate infections. Oral therapy is continued for at least 6 months in disseminated or pulmonary disease.

Cryptococcosis

Cryptococcosis is caused by the encapsulated yeast *Cryptococcus neoformans*. Immunocompetent individuals usually develop mild or covert disease; however, some develop pulmonary cryptococcosis. It is a major source of morbidity and mortality in patients with AIDS. Infection occurs via inhalation of the yeast, which is typically found in soil contaminated with pigeon/bird droppings. Pulmonary disease presents with cough, hemoptysis, and chest pain. Chest radiographs might demonstrate a solitary nodule, focal infiltrates, or diffuse infiltrates. Diagnosis is confirmed via isolation of the organism from body fluid or tissue. Encapsuated yeast cells will be seen with India ink staining. The treatment consists of amphotericin B alone or in combination with flucytosine for serious infections.

Coccidioidomycosis

Coccidioidomycosis is a systemic mycosis caused by the dimorphic fungus *Coccidioides immitis*. *C. immitis* is endemic in the southwestern United States, northern Mexico, and certain areas of Central and South America. Most individuals residing in endemic areas have experienced an infection with *C. immitis*. Approximately 5% of primary infections present as a flulike illness with fever, headache, malaise, cough, and anorexia. Pulmonary infiltrates might be noted on chest radiograph. Most individuals resolve their primary infection without complications; however, disseminated disease occurs in less than 1% of individuals and affects the skin, bones, joints, meninges, and genitourinary system. Those at risk for disseminated disease include pregnant women, African Americans, Filipinos, neonates, the elderly, and those in an immunocompromised state. Diagnosis is confirmed via an IgM response detected by latex agglutination, enzyme immunoassay, immunodiffusion, or tube precipitin. Antifungal therapy is not indicated in an uncomplicated primary infection. Amphotericin B is recommended for severe infections.

Histoplasmosis

Histoplasmosis is caused by any number of *Histoplasma capsulatum* varieties (*H. capsulatum* var. *capsulatum*, *H. capsulatum* var. *duboisii*, and *H. capsulatum* var. *farciminosum*). *H capsulatum* is found in soil containing high nitrogen concentrations enriched by bird (especially pigeons) droppings. Infection is acquired via inhalation of microcondidia. Most infections with *H. capsulatum* are asymptomatic (95%) or present with mild upper respiratory tract symptoms. The most common symptomatic illness is pulmonary disease, which manifests as a flulike illness with fever, cough, headache, and chest pain. Chest radiograph reveals patchy infiltrates with an alveolar parenchymal pattern with associated hilar/paratracheal lymphadenopathy. The disease typically resolves without treatment in several weeks. Individuals with chronic obstructive pulmonary disease can develop chronic cavitary histoplasmosis Disseminated disease is rare in immunocompetent hosts. Culture is the definitive method of diagnosis. Most cases of histoplasmosis are self-limited, but chronic cavitary or disseminated disease requires treatment. Currently, amphotericin B, miconazole, and ketoconazole are chemotherapeutic options.

Chronic mucocutaneous candidiasis

Chronic mucocutaneous candidiasis (CMC) is a rare heterogenous primary immune deficiency disorder characterized by recurrent and chronic *Candida* infections of the skin, mucous membranes, and nails with *C. albicans*, *C. parapsilosis*, *C. tropicalis*, or *C. guilliermondii*. Typically, the disease begins in infancy or childhood, and persistent oral thrush is the most common presentation (followed by chronic diaper dermatitis in infants). Both autosomal recessive and autosomal dominant modes of inheritance have been reported.

The immunologic defect in CMC is a thymus-dependent T-cell dysfunction. Abnormal delayed cutaneous hypersensitivity with anergy to intradermal *Candida* skin test is commonly found. However, CMC has also been described in patients with normal delayed cutaneous response to *Candida*. It is believed that most individuals with CMC have normal neutrophil, monocyte, and complement function. Elevated serum IgG and IgA levels are frequently seen along with elevated anti-*Candida* antibody titers.

In nearly 50% of the patients, CMC is associated with various endocrinopathies, especially if the CMC is autosomal recessive. Autosomal dominant CMC is rarely associated with endocrinopathies. Hypoparathyroidism is the most common defect seen in CMC, but others, including Addison's disease, hypothyroidism, hypopituitarism, pernicious anemia, diabetes mellitus, and gonadal failure, also can occur. Such patients are also at increased risk for autoimmune diseases including immune thrombocytopenic purpura, neutropenia,

alopecia, juvenile rheumatoid arthritis, and hemolytic anemia. Thymoma commonly occurs in patients who develop CMC later in life.

The diagnosis of CMC is based on persistent mucous membrane and/or cutaneous *Candida* infections for at least 2 years and the lack of general immunosuppression. Ketoconazole is the treatment for CMC as either intermittent or chronic therapy. Numerous experimental treatment options have been described, including fetal thymic tissue transplants, human leukocyte antigen (HLA)-matched leukocyte transfusions, transfer factor infusions, and immunostimulatory agents; however, pharmacotherapy is recommended [19].

Toxin-induced Disease

Mycotoxicosis

"Toxic mold syndrome" is a controversial diagnosis and refers to a constellation of nonspecific systemic symptoms in persons exposed to mold. Symptoms reported include headache, rhinorrhea, cough, dyspnea, epistaxis, diarrhea, nausea, vomiting, memory loss, generalized and focal weakness, and mood variations. There is currently a lack of objective evidence linking mold exposure to these nonspecific symptoms cited by patients. Recently, there has been a media frenzy related to reports of mold growth amplification in homes, schools, and office environments causing numerous nonspecific systemic effects caused by mycotoxin exposure following water incursion or "tight" buildings. Numerous lawsuits have been filed by individuals for alleged ill effects caused by exposure of molds and mycotoxins.

Historical perspective

For centuries, it has been known that certain fungi produce toxic metabolites that cause biologic responses in man. In the 1700s, ergot poisoning, one of the first known mycotoxicoses, occurred after the ingestion of rye and millet contaminated with *Claviceps*. *Claviceps* is a parasitic fungus that produces toxic alkaloids. Epidemics of ergot poisoning occurred in two forms, gangrenous and convulsive. In the gangrenous form, victims suffered burning pains known as "St. Anthony's fire," with subsequent numbness and necrosis of limbs. In the convulsive form, persons experience seizures and death.

Mycotoxins

Mycotoxins are low molecular weight chemicals that are produced by fungi during the process of metabolizing nutrients. The word mycotoxin is based on the Greek words "μυκης," which means fungus, and "τοξικον," which means arrow poison. They are secondary metabolites and have no known physiologic function. Their toxic effects are called "mycotoxicoses." Mycotoxin production is a complicated and poorly understood process that depends on numerous factors, including temperature, moisture, nutrition, substrates available for growth, the genetic pattern, the maturity of the specific fungal colony, and competition from other organisms. Also, the types and amounts of mycotoxins produced can vary greatly from one isolate to another. Therefore, even if a toxic mold species is present, it cannot be presumed that it is or will produce mycotoxins [20••].

Mycotoxins can elicit effects via inhalation, ingestion, or direct skin contact. They are not significantly volatile, nor do they evaporate. An inhalation exposure would require production of an aerosol of fungal spores, mycelial fragments, or contaminated substrates. Spores are typically the most common inhalation exposure unit for mycotoxins. The amount of toxin per spore has been reported to be between 0.02 nanograms per 10^6 spores and 9.9 nanograms per 10^6 spores. Therefore, massive spore exposure containing high levels of toxin would be necessary to elicit symptoms in humans. Levels higher than 10^6 spores per m^3 of air for short periods or constant exposure to more than 1000 spores per m^3 for several days are considered a toxic exposure [21•]. These extremely high levels of exposure in homes, offices, and schools are rare, and occur primarily in the farming, swine, and poultry industries. They have been associated with Organic Dust Toxic Syndromes.

Organic Dust Toxic Syndromes are a heterogeneous group of lower respiratory disorders typified by transient cough, dyspnea, headache, nausea, fever, chills, malaise, and myalgia. Pulmonary function is minimally affected, and chest radiographs are usually normal. The triggers seem to be toxins or organic gases. Symptoms occur in most exposed individuals, especially those with high exposures. Episodes have occurred in situations with "thick airborne dust" that "worsened until it was no longer possible to see across the room" [22]. Total spore counts have ranged from 10^5 to 10^{10} spores per m^3 of air [23,24].

Mycotoxins are commonly measured in foodstuffs worldwide. However, it is difficult to accurately and precisely determine the mycotoxin concentration in large bulks of food owing to wide variability in the sampling and preparation of the test product. Recent reports have focused on measuring mycotoxins in patients and their environment. The detection of polar and macrocyclic trichothecene mycotoxins from air samples of a mold-contaminated building [25,26] and from a mold-exposed patient's urine [27] have been reported, but these methods need further evaluation and validation.

More than 300 mycotoxins have been identified, and many have been implicated in human disease. Table 3 provides a partial list of mycotoxins, their respective fungal source, and the associated illness based on human ingestion, animal ingestion data, or animal exposure [28••]. Documentation of a connection between inhalation of mycotoxin and human disease has not been forthcoming.

Specific Mycotoxins

Aflatoxin

Aflatoxins are produced by *Aspergillus flavus* and *Aspergillus parasiticus* on agricultural commodities. Aflatoxins identified are designated B_1, B_2, G_1, G_2, M_1, and M_2. Typically these toxins are found in areas where climatic conditions are con-

Table 3. Mycotoxins associated with foods, feeds, and environmental sources

Mycotoxin	Fungus	Effected subjects	Source	Symptoms
Aflatoxins	*Aspergillus flavus*	Humans, dogs, swine	Corn	Anorexia, weight loss, tremor, ataxia, liver failure, death
Clavine alkaloids	*Claviceps fusiformis*	Humans, camels	Millet	Nausea, vomiting, giddiness, drowsiness
Ergometrine	*C. purpurea*	Humans	Barley	Weakness, burning sensation, vomiting, diarrhea, limb swelling, gangrene
Fumonisins	*Fusarium verticillioides*, *F. proliferatum*	Horses, swine	Corn	Equine leukoencephalomalacia, porcine pulmonary edema, cyanosis, death
Cyclopiazonic acid	*A. flavus*, *A. tamarii*	Humans	Millet	Nausea, giddiness
Ochratoxin A	*Penicillium verrucosum*, *A. ochraceus*	Human, swine	Grains, dried vegetables, meat, fish, olives, wheat silage dust	Interstitial nephropathy, edema
T-2 toxin	*F. tricinctum*	Cattle	Corn	Abortion, visceral hemorrhage, death
Trichothecenes	*Fusarium* and *Aspergillus* spp.	Humans	Wheat	Vomiting, diarrhea, bloody stools, and abdominal pain
Tremorgens	*A. fumigatus*, *A. niger*, *A. flavus*, *A. clavatus*, *Mucor*, *Rhizopus*, *Penicillium*, *Cephalosporum*	Humans	Clover grass and alfalfa silage dust	Fatigue, fever, chills, vomiting, dementia, tremor
Slaframine, swainsonine	*Rhizoctonia leguminicola*	Horses, cattle, sheep	Red clover, wheat, and grass	Profuse salivation, lacrimation, diarrhea, increased urination, joint stiffness, tremor, abortion, increased work of breathing, death

ducive to mold growth, and primitive methods are used for storage and harvesting, as in Asia and Africa. Aflatoxin-associated animal disease was first recognized in the 1960s when contamination of peanut meal was identified in the death of more than 10,000 turkeys (Turkey X disease) [29]. Since then, aflatoxins have been identified as teratogenic and hepatocarcinogenic in animals [30], and an association between aflatoxin ingestion and the development of human hepatocarcinoma has been established [31]. Currently, the US Food and Drug Administration monitors and regulates the level of aflatoxin contamination in feed grain, milk, and eggs.

Ochratoxicosis

Aspergillus ochraceus and *Penicillium verrucosum* are the two major fungi that produce ochratoxin A. Ochratoxin A is produced primarily in cereal grains during storage in temperate climates. However, it has been found in coffee, nuts, cheese, fish, wine, beer, beans, and milk powder [32]. Ochratoxin A is a potent nephrotoxin in animals [33]; its association in human disease is speculative. In the late 1950s, outbreaks of kidney disease occurred in rural Bulgaria, Romania, Tunisia, and the former Yugoslavia that were associated with ochratoxin A contamination of grain. Currently, eight countries monitor foodstuffs for levels of ochratoxin A.

Stachybotryotoxicosis

Stachybotrys chartarum, also known as *S. atra* and *S. alternan* is a filamentous fungi capable of producing several mycotoxins under certain environmental conditions. This greenish-black/sooty-appearing cellulolytic saprophytic fungus is distributed worldwide. *S. chartarum* is commonly found in areas rich in cellulose, such as hay, cereal grains, wood, paper, cotton, and plant debris. The spores are produced in humid conditions in which the moisture content of the substrate is more than 15% and the relative humidity is greater than 90%. The spores are not easily airborne. *Stachybotrys* does not compete well with other common molds, including *Penicillium* and *Aspergillus*, and is rarely found in outdoor air. Indoor levels are typically low, even with extensive surface mold growth, owing to its poor ability to compete with other molds [34].

Stachybotrys produces more than 40 different toxins, including trichothecenes (*ie*, satratoxin), spirocyclic lactones, and stachybotrylactams. A recent review article [35] summarized *Stachybotrys's* mycotoxin nonhuman biologic effects as: 1) inhibiting protein synthesis in plant/animal models; 2) altering glycolysis, the pentose phosphate pathway, and the tricarboxylic acid cycle; 3) inducing hemolysis with sheep's blood; 4) disrupting development of fetal rabbit alveolar II cells; 5) disrupting interleukin (IL)-2 production and viability in mouse T cells; 6) reducing cholesterol absorption and total cholesterol in rats; 7) inhibiting the complement cascade; 8) reducing natural killer cell activity; and 9) reducing antibody-dependent cell-mediated cytotoxicity. Nikulin *et al.* [36] reported that intranasal instillation of *S. atra* spores containing satratoxin resulted in severe intra-alveolar, bronchiolar, and interstitial inflammation with pulmonary hemorrhage in mice. The effects of passive inhalation of *Stachybotrys* were investigated in a mouse model. Mice were placed in a cham-

ber with *S. chartarum* at a level equivalent to that which would cover all surfaces of a room. Despite airflow stronger than four times the typical level in a home, no pulmonary effects were documented [37].

Stachybotrys has been associated with toxicity, or stachybotryotoxicosis, via several routes, including contact, ingestion, or inhalation. The toxic effects of *S. chartarum* were first documented in the 1920s in Russia. Researchers noted that cattle and horses that ingested hay contaminated with the mold developed significant symptoms, including mucous membrane bleeding, diarrhea, upper/lower respiratory disorders, and skin disease. Many animals died within weeks after the onset of the disease [38].

Dermatitis of the axilla, scrotum, and hands has been reported in individuals who handle fodder or sleep on mold-infested straw mattresses. Fingertip-skin inflammation, consisting of a painful rash followed by scaling, was reported in several women handling pots made from recycled paper contaminated with *Stachybotrys*. It was not determined whether the etiology was irritant, allergic, or toxic [39].

Stachybotrys was implicated as a causative agent of pulmonary hemorrhage in several case reports [40–42] and in more than 30 infants in the Cleveland area during the 1990s. In Cleveland, a case-control study determined that the affected infants were more likely than control infants to have lived in water-damaged homes in which *Stachybotrys* was isolated [43]. The investigation concluded that *Stachybotrys* was likely responsible for the pulmonary hemorrhage in the infants, owing to the increased vulnerability of their rapidly growing lungs and the exposure to tobacco smoke (90% of cases). However, in 1997, a re-evaluation of the initial study by the Centers for Disease Control (CDC) suggested that there were errors in calculating the mean *S. chartarum* concentration in each house; the air sampling environmental conditions and techniques were not standardized, and improper statistical matching of controls and cases had occurred. The CDC concluded that the evidence from the original studies was not sufficient to support an association between *S. chartarum* and acute idiopathic pulmonary hemosiderosis [44]. Although the causality of mold and pulmonary hemorrhage remains controversial, in 1998 the American Academy of Pediatrics recommended that young infants should not be exposed to chronically moldy environments, and that, in infants with idiopathic pulmonary hemorrhage, the parents should be questioned about water damage to their home environment.

Recently, numerous reports have implicated *Stachybotrys* mycotoxins as causative agents for multiple symptoms in water-damaged buildings. Johanning *et al.* [45] reported that *Stachybotrys*, among other molds, were responsible for numerous self-reported, nonspecific symptoms of fatigue, dermatitis, and lower respiratory tract symptoms in workers of a water-damaged, mold-contaminated building. Immune dysfunction was considered, but the only significant laboratory test finding was a difference between the T lymphocytes (controls [76%] and cases [74%]) in 20 hematologic/immunologic studies. The conclusions might be called into question because the investigators did not test the control subject's home/work environment for mold, and the difference in T lymphocytes could have been laboratory variance. Similarly, Hodgson *et al.* [46] loosely associated "inhaled fungal toxins" from *Aspergillus* and *Stachybotrys* as causes of asthma, emphysema, and interstitial lung disease in workers at a flooded courthouse. The study findings were weakened by a lack of physical findings, the presence of normal chest radiographs, and no reliable biomarkers for mold exposure. Other publications have associated neurobehavioral and respiratory symptoms [47] and multiple symptoms ranging from depression to headache [48] to *Stachybotrys* toxins, but, again, these studies lack appropriate controls.

Sudakin [47], Burge [21•], and Terr [35] have published reviews discussing *Stachybotrys* and its current role in disease. Overall, despite numerous animal-model and case-control studies, proof is lacking to demonstrate that exposure to *S. chatarum* in home, school, or office environments is a cause of human disease. Randomized, controlled trials are needed to further investigate the effects of *S. chatarum* on human health.

Conclusions

In conclusion, fungi are omnipresent in our world. Although molds are common and an important allergen in IgE-mediated disease, the non-IgE manifestations, such as infections or hypersensitivity pneumonitis, are unusual. Currently, there is no scientific evidence to support the allegation that human health is affected by inhaled mycotoxins. However, if mold is discovered in a home, school, or office, the source should be investigated, and appropriate remediation undertaken to minimize structural damage and potential allergic sensitization.

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23

Hypersensitivity Pneumonitis

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Pearls for Practitioners

Roy Patterson

- Hypersensitivity pneumonitis should be considered in chronic or acute noninfectious pneumonitis. It is uncommon but important since lung destruction can occur.
- The list of potential causes is long. Just run through the list. Most of them can be excluded by using common sense.
- Keeping the causative source of antigen in the home is dangerous as lung destruction progresses. Deaths have resulted from prolonged exposure to birds in the house.
- The diagnosis of hypersensitivity pneumonitis in a worker where litigation is involved can be a real problem.
- Some people would rather risk lung destruction than give up their birds.
- Treating a patient with corticosteroids when the antigen remains in the environment is dangerous.
- Unfortunately, serodiagnosis by some laboratories results in false-negative results. Know the laboratory you use.
- Positive prescriptions against antigens may only indicate exposure, not hypersensitivity pneumonitis. Clinical skill is required.

Most of the hypersensitivity diseases of the respiratory tract in humans are asthma or rhinitis, and are caused by the release of pharmacologic mediators from mast cells and the recruitment of inflammatory cells as a result of IgE antigen-initiated reactions. The inhaled antigens usually are common pollen grains, mold spores, or animal proteins, and the resulting reaction induces bronchospasm, mucosal edema, increased secretions, and inflammation. However, allergic respiratory reactions may take other forms, and additional immunologic processes involving precipitating antibodies, circulating antigen–antibody complexes, and cellular mechanisms may play a role in the pathogenesis of the disorders. Although asthma and rhinitis occur most often in individuals with atopic constitutions, the diseases discussed in this chapter can be seen in both atopic and nonatopic patients. The disorders may be grouped under the general term of *hypersensitivity pneumonitis* but are also referred to as *extrinsic allergic alveolitis*. These diseases occur as the result of immunologic inflammation after the inhalation of any of several organic dusts.[1–25] They present in several clinical forms, depending on the patient's immunologic responsiveness and intensity of exposure to the offending dust, as well as the antigenicity of the inhaled biologic dust.

ETIOLOGY

Almost any inhaled organic dust can sensitize and result in the development of hypersensitivity pneumonitis; a list of antigenic materials associated with the disorders is shown in Table 23-1. The diameters of the inhaled particles that reach the terminal airways where lesions are initiated are no larger than 3 to 5 μm. The dusts may be derived from animal proteins; for example, in pigeon breeder's disease, the inhaled antigens are contained in dried avian droppings [3,13,23] In pituitary snuff-taker's disease, the offending material is the pituitary powder containing bovine or porcine proteins The inhalation of vegetable dusts contaminated with various microorganisms also causes hypersensitivity reactions such as farmer's lung, bagassosis, and mushroom picker's disease In these disorders, the inhaled dusts from the moldy vegetation are contaminated with thermophilic actinomycetes such as *Micropolyspora faeni*, *Thermoactinomyces vulgaris*, *Thermoactinomyces viridis*, or *Thermoactinomyces candidus*, whose spores are smaller than 1 mm and can reach terminal airways, causing sensitization and subsequent immunologic inflammatory lung disease These thermophilic bacteria are ubiquitous and grow best at temperatures of 45° to 50°C, which commonly occur in decomposing hay, sugar cane, or mushroom compost. Thermophilic actinomycetes also have been

TABLE 23-1. *Sensitivity materials in hypersensitivity pneumonitis*

Etiology	Disease entity	Antigenic material inhaled	antigen
Induced by serum proteins	Bird breeder's lung	Avian dust	Avian proteins
	Pituitary snuff taker's lung	Pituitary powder	Bovine or porcine proteins
	Pearl oyster shell pneumonitis	Pearl oyster shell dust	Oyster shell glycoprotein
Induced by microorganisms	Farmer's lung	Moldy hay	*Micropolyspora faeni* or *Thermoactinomyces vulgaris*, *T sacchari*, *T viridis*, *T candidus*, *Micropolyspora* sp
	Bagassosis	Moldy sugar cane	
	Mushroom picker's lung	Mushroom compost	
	Pneumonitis from contaminated air conditioner, humidifier, or heating system (forced air system disease)	Dust from air conditioners, humidifier, or furnace	
	Maple bark disease	Moldy maple bark	*Cryptostroma corticale*
	Sequoiosis	Redwood dust	*Graphium* sp
	Suberosis	Moldy cork dust	*Penicillium frequentans*
	Cheese washer's lung	Cheese particles	*Penicillium caseii*
	Paprika splitter's lung	Paprika dust	*Mucor stolonifer*
	Malt worker's lung	Malt dust	*Aspergillus clavatus*
	Summer pneumonitis	House dust	*Trichosporon cutaneum*
Similar diseases	Smallpox handler's lung	Smallpox scab dust	Unknown
	Enzyme worker's lung	Enzyme dust	*Bacillus subtilis*
	Bathtub refinisher's lung	Chemical catalyst	Toluene diisocyanate
	Epoxy resin lung	Heated epoxy resin	Phthalic anhydride
	Plastic worker's lung	Plasticizer	Trimellitic anhydride
	Drug induced	Pharmacologic agents	Gold, thiazides, penicillin, tetracyclines, hydroxyurea

shown to contaminate forced air heating, humidification, or air-conditioning systems of commercial or residence buildings where hypersensitivity pneumonitis can occur as a result of sensitization to these and other contaminants.[2,12]

The inhalation of other antigens also may result in hypersensitivity pneumonitis. Workers removing the bark from maple logs and inhaling the spores of *Cryptostroma corticale*,[11] woodworkers exposed to redwood dust that contains the mold *Graphium* sp,[8] individuals working in cheese factories in which *Penicillium caseii* spores may be inhaled, and brewers working in malt factories where spores of *Aspergillus clavatus*[21] may be present also develop disease. A similar pneumonitis also may occur in workers exposed to the enzyme of *Bacillus subtilis* used in detergent manufacturing,[14] and after exposure to organic chemicals such as phthalic anhydrides[24] or toluene diisocyanate.[25] Recently, pneumonitis occurring only in the summer in Japan has been traced to homes contaminated with *Trichosporon cutaneum*.[13] The list of inhaled organic dusts that result in a hypersensitivity pneumonitis grows as exposure to new antigens increases.

In addition to the presence of an organic dust in the environment, other factors play a role in the development of a hypersensitivity pneumonitis in the exposed individual. The frequency and extent of exposure as well as the immunologic reactivity of the host are likely to influence the response to the dust, as are factors such as ciliary transport mechanisms, alveolar macrophage phagocytosis, and other coexisting pulmonary inflammatory processes.

CLINICAL FEATURES

The clinical manifestations of these respiratory disorders may present in several forms, depending on the immunologic response to the inhaled antigen, the antigenicity of the dust, and the frequency and intensity of exposure (Table 23-2). In general, the manifestations are similar, regardless of organic dust inhaled, and hypersensitivity pneumonitis may be considered as a syndrome with a spectrum of clinical features, although each specific disease may be caused by a different organic dust. The atopic individual may demonstrate typical bronchospasm or rhinorrhea immediately after inhalation of the dust; this reaction may be followed hours later by clinical features of a hypersensitivity pneumonitis. The nonatopic patient, however, usually responds with the late-type reaction characteristic of these disorders.

TABLE 23-2. *Clinical features of hypersensitivity pneumonitis*

Feature	Acute form	Subacute form	Chronic form
Relation of symptoms to exposure	+	+	−
Chills, fever	+	±	−
Cough, dyspnea	+	+	+
Malaise, myalgia, arthralgia	+	+	±
Anorexia, weight loss	±	+	+
Interstitial rales heard	+	+	±
Clubbing	−	−	±
Chest x-ray	Nodular infiltrates	Nodular infiltrates	Fibrosis honeycombing
Pulmonary function	Restriction	Restriction	Restriction or obstruction
Serum precipitins	+	+	+
Reversible with avoidance	Rapid	Slow	None
Reversible with corticosteroids	Rapid	Rapid	None

Acute Form

The most common and most easily recognized form of hypersensitivity pneumonitis follows intermittent exposure to a specific organic dust. Within 4 to 6 hours of exposure, the sensitized patient develops symptoms of cough, dyspnea, fever, chills, myalgia, and malaise, resembling a systemic viral or bacterial infection. The symptoms persist for 8 to 12 hours, but the patient recovers spontaneously, only to experience a recurrence of symptoms with reexposure. Numerous attacks may be associated with weight loss and anorexia. Between the acute attacks and in the absence of further antigen exposure, the patient often feels normal. Clinical examination during an attack reveals an acutely ill, dyspneic patient with prominent bibasilar moist rales. Although the patient appears to recover within a few hours, the rales may persist for a few days.

During the attack, laboratory studies usually demonstrate a leukocytosis with the white blood cell count as high as 25,000. Eosinophilia is unusual but may be as high as 10%. Often, levels of total serum IgG are elevated, but in some patients all of the major immunoglobulin classes are increased. Levels of IgE are elevated only in patients with atopic diseases; IgE levels usually are normal in hypersensitivity pneumonitis.

Results from pulmonary function studies done during the asymptomatic period of the acute form of a hypersensitivity pneumonitis usually are normal. Measurable changes occur 4 to 6 hours after exposure to the offending antigen (Fig. 23-1). There is a reduction in vital capacity, a decrease in gas transfer across the alveolar wall (as measured by diffusing capacity), and a

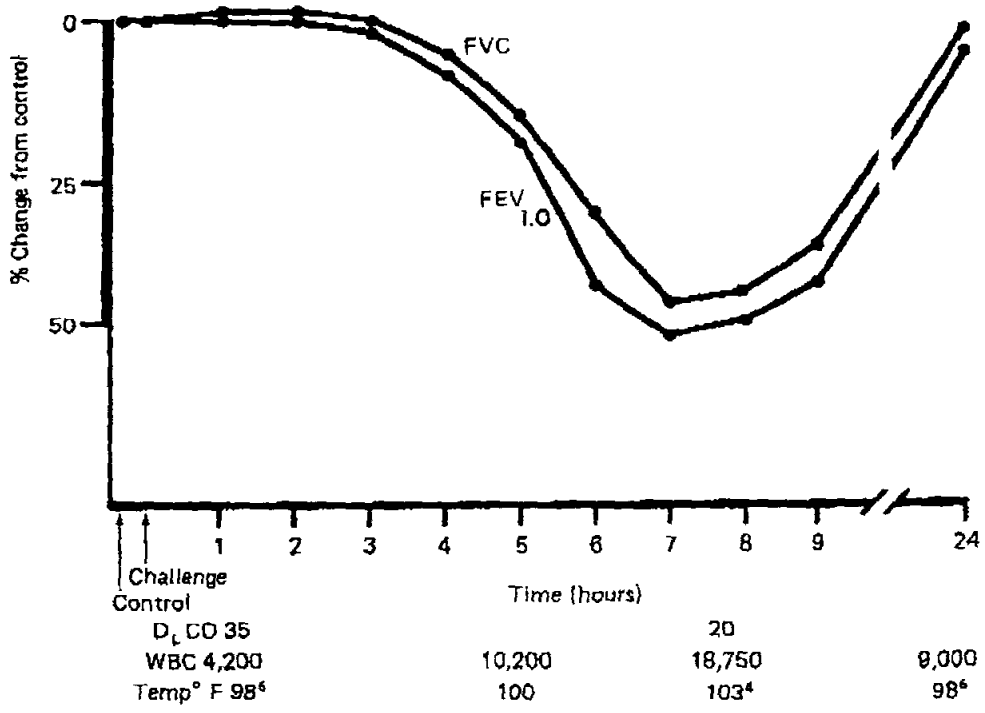

FIG. 23-1. Pulmonary function changes in an acute episode of hypersensitivity pneumonitis. Notice the time delay from challenge. FVC, forced vital capacity; $FEV_{1.0}$, forced expiratory volume in 1 second; DL_{CO}, diffusion capacity.

decrease in pulmonary compliance. Some patients also demonstrate decreases in expiratory flow rates and 1-second forced vital capacity, indicating airway obstruction. Chest x-rays may show fine nodular densities and peripheral infiltrates suggestive of interstitial and alveolar involvement (Figure 23-2), but a normal chest roentgenogram finding does not exclude the disease. With avoidance of exposure to the offending materials or therapy with corticosteroids, all symptoms disappear and abnormal laboratory test results return to normal. Continued intermittent exposure to the offending organic dust, however, may lead to permanent pulmonary function and radiographic abnormalities associated with progressive respiratory insufficiency. Fatalities because of progressive hypersensitivity pneumonitis have been reported.[26,27]

Subacute Form

Some patients have a more insidious type of disease with rare acute attacks. These individuals usually are exposed to small amounts of antigen over long periods (e.g., lovebird or parakeet fanciers). The symptoms resemble those of a progressive bronchitis: dyspnea, chronic productive cough with scanty sputum, anorexia, fatigue, and weight loss. Pulmonary function

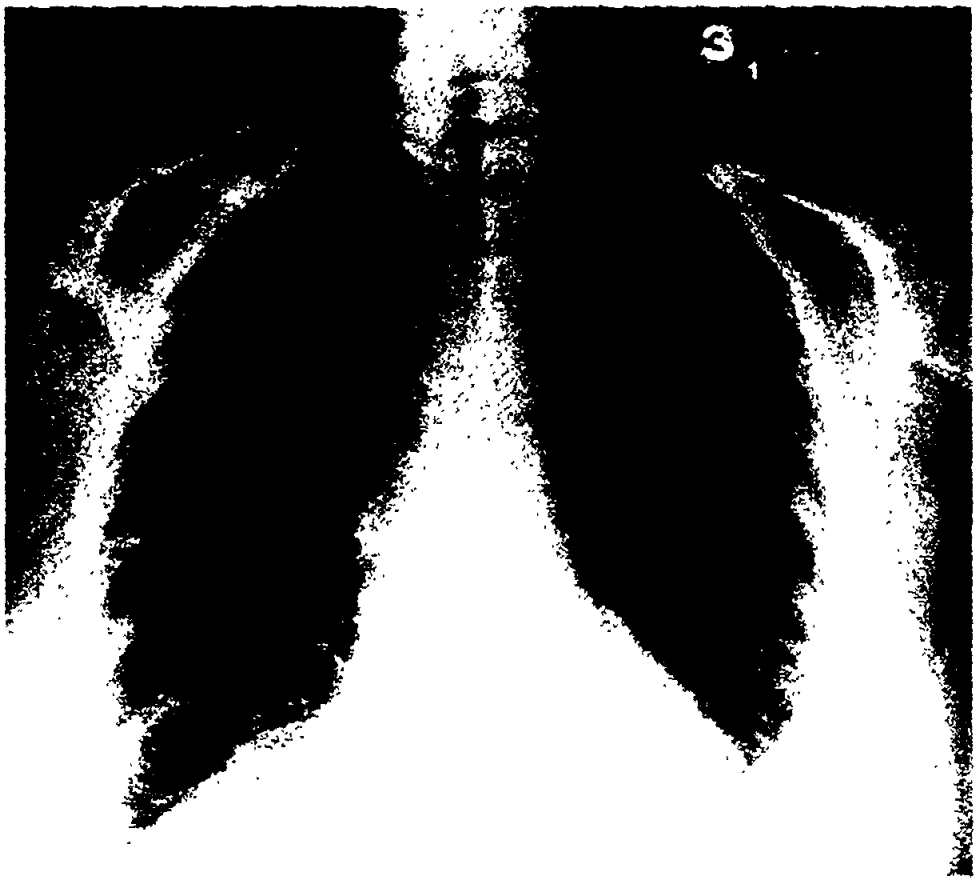

FIG. 23-2. Chest x-ray of patient with a hypersensitivity pneumonitis caused by the inhalation of thermophilic actinomycetes contaminating her furnace humidifier

abnormalities of progressive restriction, diffusion defect, and increased stiffness of the lung are seen. These patients often are diagnosed as having chronic bronchitis, recurrent episodes of influenza, idiopathic pulmonary fibrosis, or Hamman-Rich syndrome. Although the clinical laboratory abnormalities respond to corticosteroids or prolonged avoidance of exposure to the offending dust, the response is much less prompt than in the acute form. If sufficient fibrosis is present, the pulmonary function abnormalities become irreversible.

Chronic Form

In some cases, chronic irreversible lung damage may occur. This may take the form of irreversible fibrosis and pulmonary insufficiency, and is seen in long-standing cases of farmer's lung and in persons who keep parakeets, budgerigars, or lovebirds. These persons have symptoms of progressive dyspnea and may develop irreversible pulmonary function abnormalities or restriction, diffusion defects, and "stiff" lungs that do not respond to corticosteroids. Lung biopsy specimens from these patients demonstrate interstitial fibrosis with granulomas and thickening of alveolar walls.

In a few patients with farmer's lung, pigeon breeder's disease, or bagassosis, pulmonary function tests show persistently marked elevation of the residual volume, diminished flow rates, and loss of pulmonary elasticity, suggestive of emphysema. Histologic examination of these lungs shows obstructive bronchitis with distal destruction of alveoli. Such patients usually do not respond to corticosteroids or avoidance of exposure, even if these measures are pursued for prolonged periods.

Although avoidance of exposure usually is followed by resolution of signs and symptoms, this does not always occur. Patients with the chronic form of hypersensitivity pneumonitis manifest progressive pulmonary impairment. Patients with multiple acute episodes of farmer's lung may continue to have respiratory impairment, even long after avoidance.[19]

IMMUNOLOGIC FEATURES

The characteristic immunologic feature of these disorders is the presence of precipitins against the offending antigen in the sera of affected individuals (Fig. 23-3). These antibodies may be demonstrated by gel diffusion techniques using the patient's serum and the suspected antigen. Immunoelectrophoresis has shown these precipitating antibodies to be of the IgG class, although other studies have demonstrated antibody activity in other classes of immunoglobulins.[28,29] A few patients have low titers of precipitins, and it may be necessary

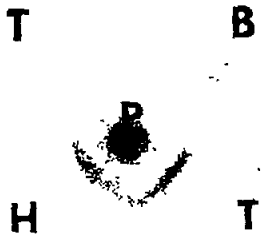

FIG. 23-3. Immunodiffusion studies of serum of patient (P) in Figure 23-2 against thermophilic antigens (T), bagasse (B), and moldy hay (H)

to concentrate their serum to detect the antibodies, but relatively high titers of these antibodies have been seen in the sera of most symptomatic patients studied. As many as 50% of asymptomatic individuals exposed to the same antigen also may have precipitins, but usually of lesser titer. Thus, the finding of precipitins must be considered in light of the clinical history when the diagnosis of hypersensitivity pneumonitis is considered.

Skin tests with suspected thermophile antigens have been shown to be unreliable because of nonspecific irritation-type reactions. In the disorders caused by inhalation of serum proteins, however, such as pigeon breeder's disease, skin tests may be of value. Both immediate wheal-and-flare and late (4- to 6-hour) skin reactions may be observed. The immediate reactions are the same type as seen with the common inhalant allergens, but the late reactions resemble the Arthus phenomenon, indicative of a vasculitis because of a precipitin–antigen reaction. The late reaction begins with a variable of edema and erythema of the injected area; it can progress to central necrosis, but it usually subsides in 24 hours unless necrosis has occurred. Histologic examination of biopsy specimens of such skin reactions has demonstrated lesions consistent with Arthus-type reactions, with a mild vasculitis consisting of polymorphonuclear and plasma cell infiltration of the vessels in the area.[1,17]

RADIOGRAPHIC FEATURES

Hypersensitivity pneumonitis cannot be distinguished radiographically from other nonimmunologic interstitial disorders. Roentgenogram findings may be normal or they may show recurrent interstitial nodular infiltrations or fibrotic changes, depending on the stage of the disease.[5,17]

PATHOLOGIC FEATURES

The histologic features of the lung in the hypersensitivity disorders depend on the stage of the disease at the time of biopsy (Fig. 23-4). In early stages of farmer's lung, bagassosis, mushroom picker's disease, and some hypersensitivity pneumonitis caused by antigens other than thermophilic organisms, the alveolar walls are infiltrated with lymphocytes. Plasma cells and histocytes containing foamy cytoplasm also may be seen within the alveolar spaces. Later, the interstitium becomes infiltrated with mononuclear cells and scattered giant cell granulomata. In still later stages, fibrosis of these areas occurs, and an organizing bronchiolitis obliterans may be seen.

In biopsy specimens from cases of pigeon breeder's disease, similar interstitial and alveolar granulomatous and infiltrative changes may be seen.[10,30,31] In addition, foamy macrophages, possibly derived from alveolar macrophages, may be found in the interstitial areas and within the alveoli. The interstitial position of these foamy cells may be unique for pigeon breeder's disease because this feature is not common in the other hypersensitivity pneumonitides.

Bronchiolitis obliterans can be observed with peripheral destruction of alveoli in some chronic cases of farmer's lung, bagassosis, or pigeon breeder's disease. The interstitial and intraalveolar infiltrate in these cases is less distinctive, and there are fewer foam-laden macrophages than in the other forms of the disorder.[30,31]

DIFFERENTIAL DIAGNOSIS

The diagnosis of a typical case of a hypersensitivity pneumonitis usually can be made by evaluating the environmental history, by examining results of appropriate laboratory and

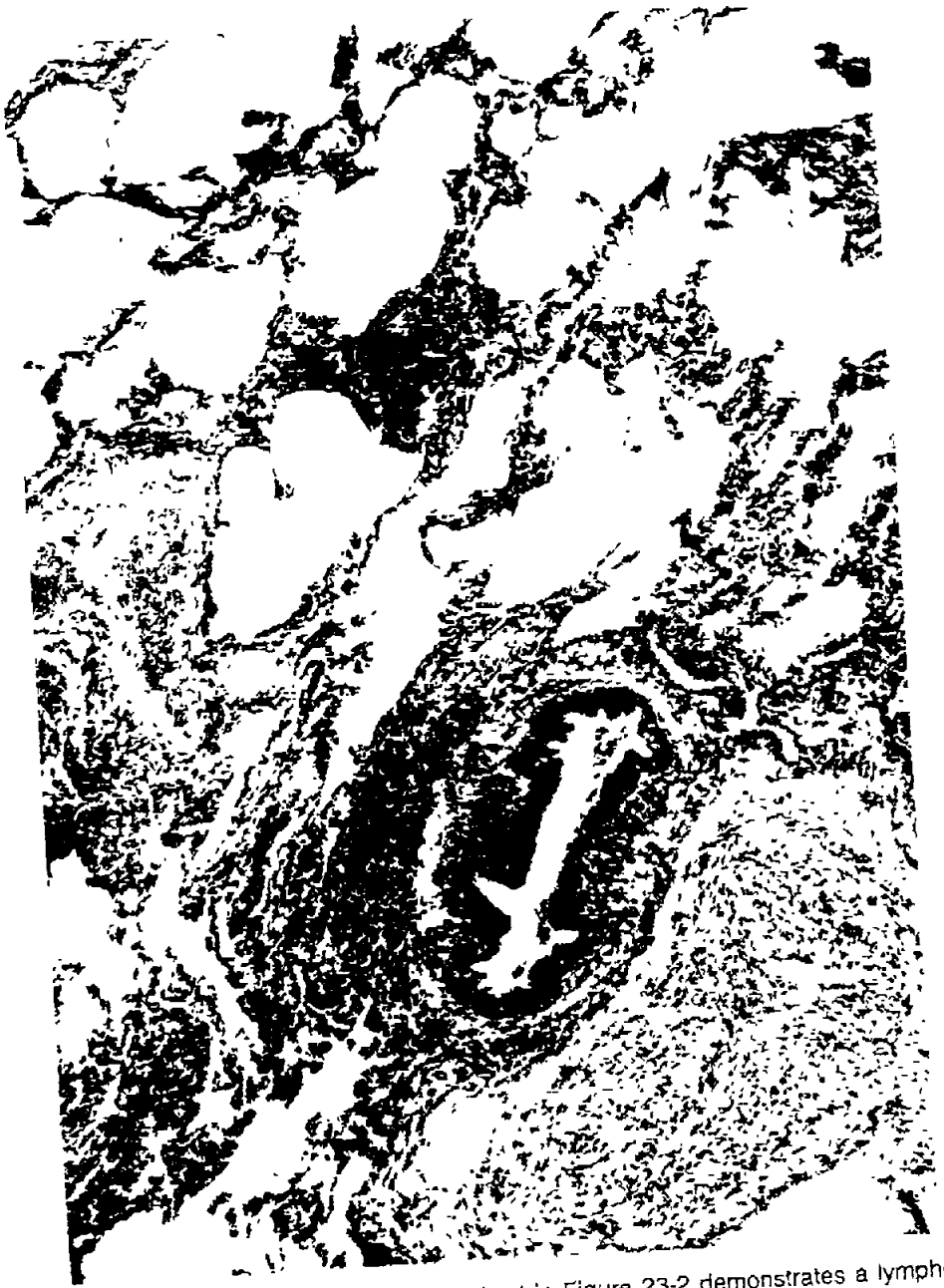

FIG. 23-4. Lung biopsy specimen of the patient in Figure 23-2 demonstrates a lymphocytic interstitial pneumonitis with early granuloma formation (×250).

serologic studies, and by using a trial of avoidance and reexposure where possible (Table 23-3). The more insidious and progressive forms of hypersensitivity pneumonitis may be difficult to diagnose. Chest x-ray and pulmonary function abnormalities of other interstitial pulmonary disorders such as chronic eosinophilic pneumonia, the collagen vascular diseases, lymphogenous spread of carcinoma, desquamative interstitial pneumonia, and sarcoid may be

similar. The finding of extrapulmonary involvement (such as generalized lymphadenopathy or abdominal organomegaly) rules out a hypersensitivity pneumonitis. At times, however, a lung biopsy may be necessary to make a definitive diagnosis. Biopsy also may be necessary to differentiate these disorders from diffuse idiopathic pulmonary fibrosis, which clinically resembles the fibrotic stage of hypersensitivity pneumonitis

CONTROLLED EXPOSURE TO ANTIGENS

On occasion, the patient may be exposed cautiously to the suspected antigen, and the reaction carefully observed. This may be done during an asymptomatic period by allowing the farmer to enter the barn or the pigeon breeder to enter the coop. The patient then should be brought to the hospital and observed frequently over the next 8 hours for symptoms or signs of hypersensitivity pneumonitis.

Some reactions may be diagnosed by careful inhalation exposure to nonirritating sterile extracts of the suspected antigen, although this is less desirable than observation after natural exposure. This purposeful exposure of an individual, however, either in the appropriate environment or by direct airway challenge, must be considered an experimental technique to be done with great caution by experienced physicians. With dilute extracts—previously shown not to induce any changes in normal individuals—minimal abnormalities in pulmonary function tests or a rise in temperature of a few degrees 4 to 6 hours after exposure may clarify the diagnosis. Corticosteroids may be needed in these cases to abort severe attacks that were induced inadvertently.

TABLE 23-3. *Diagnostic methods of hypersensitivity pneumonitis*

History
Suspicious of environmentally induced symptoms
Chest X-Ray
Abnormalities depend on stage of disease and proximity to exposure
Normal finding on chest x-ray is possible
Pulmonary Function
Abnormalities depend on stage of disease and proximity to exposure
Usually ↓FVC, ↓DLCO, ↓Pao_2
Normal function is possible
Precipitins
Usually present to offending environmental agents
Trial of Avoidance
Results in relief of symptoms and return to normal function
Bronchoalveolar Lavage
Lymphocytosis with predominance of suppression T cells
Lung Biopsy
Features characteristic of hypersensitivity pneumonitis

FVC, forced vital capacity; DLCO, diffusion capacity of the lungs for carbon monoxide; Pao_2, arterial partial pressure of oxygen.

PATHOGENESIS

Evaluation of pulmonary cell populations from patients with hypersensitivity pneumonitis has revealed activation of macrophages and release of proinflammatory cytokines and CD8 or suppressor cell attractants from those cells.[32,33] Further, the alveolitis of hypersensitivity pneumonitis is lymphocytic and is largely composed of CD8 suppressor cells, which appear to have decreased functional capacity.[34–38] The results of these processes likely are the major pathogenetic mechanisms of the inflammatory response seen in this disease

THERAPY

As in all other allergic disorders, the primary therapy should be avoidance of the offending antigen once it is known. Because many of these disorders are occupational, certain measures may be necessary, such as the use of masks with filters capable of removing the antigen, appropriate ventilation of working areas, or even a change of occupation.

Drug therapy may be needed in the acute or subacute forms of these disorders when avoidance cannot be carried out immediately. Although antihistamines or bronchodilators have no effect on the symptom pattern, patients usually respond to the administration of corticosteroids. Moderate doses of these drugs may be necessary for prolonged periods, along with avoidance, to determine if reversibility of the clinical abnormalities is possible. Hyposensitization should be avoided because toxic immune complexes may be formed when the injected antigen combines with the precipitating IgG, and systemic vasculitis or serum sickness may result.

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IN THE UNITED STATES DISTRICT COURT
FOR THE SOUTHERN DISTRICT OF GEORGIA
SAVANNAH DIVISION

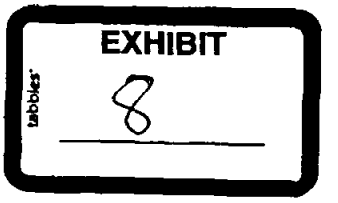
CHRIS JAZAIRI, Plaintiff. VS. ROYAL OAKS APARTMENT ASSOCIATES, L.P., Its Parent Company And Subsidiaries, And MITCHELL L. MORGAN MANAGEMENT, INC., Defendants.	)	CIVIL ACTION FILE NO.: CV-04-404-091

STATE OF NEW YORK)
)
COUNTY OF Albany)

AFFIDAVIT OF ECKARDT JOHANNING, M.D.

1. Affiant is of legal age, gives this affidavit based on personal and professional knowledge and understands that this affidavit will be submitted in the action of *Chris Jazairi v Morgan Mitchell Properties, et al.*, U.S. District Court, Southern District of Georgia, Savannah Division, Civil Action No. CV-04-404-091.

2. Affiant understands that his differential diagnosis of mold induced lung inflammatory injury of Chris Jazairi has been criticized, in part, due to the lack of published human mold spore exposure standards. Molds are living organisms and they release mold spores into the air. These mold spores may be inhaled by humans. The mold spores are biological



aerosols, which can be referred to as bioaerosols. Biological materials are different from chemicals, including pharmaceutical drugs, or dusts, such as asbestos.

3. There are few published threshold limit values [TLV] for limited number of Bioaerosols, although current professional consensus is that no validated "threshold" levels exist and can exist, because of methodological limitations and the natural properties of bioaerosols. See Exhibit 1. ACGIH TLV Statement on Bioaerosols: American Council of Government Industrial Hygienists Presented For the Bioaerosols Committee by Harriet M. Ammann, Ph.D. at 520-22. For purposes of the ACGIH guidelines, indoor microbial growth is considered a bioaerosol. Id.

4. A general TLV for cultural and countable bioaerosol concentrations is not scientifically supportable because of the complex mixtures of bioaerosols, individual human variability in response to exposures and the difficulty of evaluating exposures with various test methods. Additionally, specific TLV's for fungal or bacterial bioaerosols have not been established to prevent in an individual hypersensitivity, allergy, and irritant or toxic exposures. Id. Although it is well known that exposure can cause hypersensitivity reactions in susceptible people, the levels at which these reactions occur differs among individuals because the reactions are allergic reactions as opposed to toxic reactions, for which some known dose-response relationship may exist.

5. The clinical evaluation has shown that Chris Jazairi has experienced an illness caused by an allergic and hypersensitivity reaction. This type reaction is primarily allergy based and different individuals would react differently to the particular combination of mold spores, that were visibly present and then confirmed by laboratory testing to exist, in Chris Jazairi's

living and working environment. There is no TLV or other published exposure standard which describes the dose at which certain individuals will develop a hypersensitivity reaction to the bioaerosol mixture in Jazairi's apartment and there is general professional agreement that any exposure therefore needs to be minimized and strictly controlled. The lack of this standard does not prevent a differential diagnosis of an injurious exposure to the mold spores in Jazairi's apartment. The medical literature that describes allergy and hypersensitivity reactions in water damaged indoor environments containing visible mold recognize that the exposure to mold spores cannot usually be quantified in the precise manner that may be required in a scientific study. However, the diagnoses of mold induced allergy and hypersensitivity is commonly accepted in the publishing medical community.

6. The physiological process of lung inflammation resulting from a mold-induced hypersensitivity is well described in the medical literature. Hypersensitivity that can be tested with immunoglobulin antibodies (i.e, IgG or IgE). This allergic reaction results in an general allergic reaction and in susceptible patients in acute or chronic inflammation of the alveoli of the lung. When the exposure is reduced, the level of IgG antibodies typically decreases and the symptoms and inflammation subside. If reexposure occurs, then the IgG antibodies typically will increase and the symptoms and inflammation may recur. Only about 10 -20% of exposed people will develop this in blood measurable hypersensitivity reaction and there is no recognized exposure dose at which this response will develop. However, some exposure must occur before the reaction can be associated with the exposure.

AND FURTHER AFFIANT SAYETH NOT.

Eckardt Johanning, M.D.

Sworn to and subscribed

before me this 11th day

of March, 2005.

Piedad Diaz

Notary Public,

Albany County, New York

(NOTARIAL SEAL)

PIEDAD DIAZ
Notary Public - State of New York
Reg. No. 01D[illegible]106165
Qualified in Albany County
My Commission Expires 03-01-08

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ACGIH TLV STATEMENT ON BIOAEROSOLS; AMERICA... COUNCIL OF GOVERNMENT INDUSTRIAL HYGIENIST... PRESENTED FOR THE BIOAEROSOLS COMMITTEE BY...

HARRIET M. AMMANN, Ph.D.

Office of Environmental Health Assessment Services, Washington State Department of... P.O. Box 47846, 7700 Cleanwater Lane, Olympia, Washington 98504-7846, Phone: 360-236-3171... 360-236-2257, e-mail: HMA0303@doh.wa.gov

Biologically Derived Airborne Contaminants. TLVs exist for certain substances of biol... origin, including cellulose; some wood, cotton, and grain dusts; nicotine; pyrethrum; ... subtilisins (proteolytic enzymes); sucrose; and vegetable oil mist. However, for the ... identified below, there are no TLVs against which to compare environmental air concentra... of most materials of biological origin. The ACGIH Bioaerosols Committee has develope... separately published guidelines to assess, control, remediate, and prevent biologically d... contamination in indoor environments.

For the purposes of the guidelines, indoor biological contamination is defined as the pr... of:

a) biologically derived aerosols of a kind and concentration likely to causes disease or ... pose people to disease;
b) inappropriate concentrations of outdoor bioaerosols, especially in buildings desig... prevent their entry; or
c) indoor microbial growth and remnants of biological growth that may become aero... and to which people may be exposed.

The guidelines define an approach to assessing and controlling bioaerosol exposu... approach relies on visually inspecting buildings, assessing occupant symptoms, ev... buildings performance, monitoring potential environmental sources, and applying pr... al judgement.

Biologically derived airborne contaminants include bioaerosols (airborne particles co... of or derived from living organisms) and volatile organic compounds that organisms ... Bioaerosols include microorganisms (i.e., culturable, non-culturable, and dead microor... and fragments, toxins, and particulate waste products from all varieties of living ... Biologically derived contaminants are ubiquitous in nature and may be modified by ... activity. All persons are repeatedly exposed, day after day, to a wide variety of such ma...

The guidelines provide background information on the major groups of bioaerosols in... their sources and health effects. The guidelines also describe methods to collect, ana... interpret bioaerosol samples from potential environment sources. Occasionally, envir... monitoring detects a single or predominating biological contaminant. More common... toring reveals a mixture of many biologically derived materials, reflecting the diverse an... active nature of indoor microenvironments.

...mental sampling for bioa... ...testable hypotheses about ... ...be exposed to bioaerosc... ...hypotheses and well-form... ...ing may be inconclusive a... ...reasons identified below, ...

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EXHIBIT
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RIAL HYGIENISTS
COMMITTEE BY

h.D.

a State Department of Health,
'846, Phone: 360-236-3171, FAX:

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Environmental sampling for bioaerosols should be conducted only following careful formulation of testable hypotheses about potential bioaerosol sources and mechanisms by which workers may be exposed to bioaerosols from these sources. Even when investigators work from testable hypotheses and well-formulated sampling plans, results from environmental bioaerosol monitoring may be inconclusive and possibly misleading.

For the reasons identified below, there are no TLVs for interpreting environmental measurements of:

a) total culturable or countable bioaerosols (e.g., total bacteria or fungi);

b) specific culturable or countable bioaerosols (e.g., *Aspergillus fumigatus*);

c) infectious agents (e.g., Legionella pneumophila, Mycobacterium tuberculosis); or

d) assayable biological contaminants (e.g., endotoxin, mycotoxin, antigens, or microbial volatile organic compounds).

A. Total culturable or countable bioaerosols. Culturable bioaerosols are those bacteria and fungi that can be grown in laboratory culture. Such results are reported as the number of colony-forming units. Countable bioaerosols are those pollen grains, fungal spores, bacterial cells, and other material that can be identified and counted by microscope. A general TLV for culturable or countable bioaerosol concentrations is not scientifically supportable because of the following:

1) Culturable microorganisms and countable biological particles do not comprise a single entity, i.e., bioaerosols in occupational settings are generally complex mixtures of many different microbial, animal, and plant particles.

2) Human responses to bioaerosols range from innocuous effects to serious, even fatal, diseases, depending on the specific material involved and workers' susceptibility to it. Therefore, an appropriate exposure limit for one bioaerosol may be entirely inappropriate for another.

3) It is not possible to collect and evaluate all bioaerosol components using a single sampling method. Many reliable methods are available to collect and analyze bioaerosol materials. However, different methods of sample collection and analysis may result in different estimates of the concentrations of culturable and countable bioaerosols.

4) At present, information relating culturable or countable bioaerosol concentrations to health effects is generally insufficient to describe exposure/response relationships.

B. Specific culturable or countable bioaerosols other than infectious agents. Specific TLVs for individual culturable or countable bioaerosols have not been established to prevent hypersensitivity, irritant, or toxic responses. At present, information relating culturable or countable bioaerosol concentrations to health effects consists largely of case reports and qualitative exposure assessments. The data available are generally insufficient to describe exposure/response relationships. Reasons for the absence of good epidemiologic data on such relationships include the following:

1. Most data on concentrations of specific bioaerosols are derived from indicator measurements rather than from measurements of actual effector agents. For example, investigators use the air concentrations of culturable fungi or represent exposure to airborne fun-

gal antigens. In addition, most measurements are from either area or source These monitoring approaches are less likely to reflect human exposure accur would personal sampling for actual effector agents.

2. Bioaerosol components and concentrations vary widely within and among differe pational and environmental settings. Unfortunately, replicate sampling is uncom bioaerosol assessments. Further, the most commonly used air sampling devices f monitoring are designed to collect "grab" samples over relatively sort time Measurements from single, short-term grab samples may be orders of magnitud or lower than long-term average concentrations and are unlikely to represent exposures accurately. Some organisms and sources release aerosols as "concen burst," which may only rarely be detected by limited grab sampling. Neverthe episodic bioaerosol releases may produce significant health effects.

C. Infectious agents. Human dose/response data are available for only a few bioaerosols. At present, air sampling protocols for infectious agents are limited an primarily for research endeavors. In most routine exposure settings, public health such as immunization, active case findings, and medical treatment, remain the defense against infectious bioaerosols. Facilities associated with increased risks mission of airborne infectious disease (e g., microbiology laboratories, animal handli ities, and health care settings) should employ engineering controls to minimize ai trations of infectious agents. Further, such facilities should consider the need for trative controls and personal protective equipment to prevent the exposure of w these bioaerosols.

D. Assayable biological contaminants. Assayable, biologically derived contaminants (e toxin, mycotoxins, antigens, and volatile organic compounds) are microbial, animal substances that can be detected using chemical, immunological, or biologic Evidence does not yet support TLVs for any of these substances. However, assay for certain common airborne antigens and endotoxins are steadily improving, and in idation of these assays is also progressing. Dose/response relationships for some ai bioaerosols have been observed in experimental studies and occasionally in epidemi surveys. Therefore, TLVs for some of these substances may be appropriate in th Also, innovative molecular techniques are becoming available for specific bioaero rently detectable only by culture or counting.

ACGIH actively solicits information, comments, and data that will help the Bi Committee evaluate the potential for health effects associated with bioaerosol expo occupational and related environments. Such information should be sent to ACGIH T Affairs office.

REFERENCE

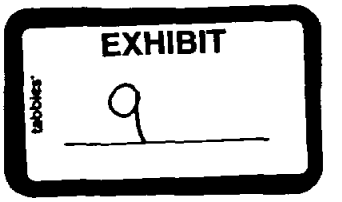
• ACGIH (American Conference of Governmental Industrial Hygienists). 1989. Gui for the Assessment of Bioaerosols in the Indoor Environment. Cincinnati, OH

IN THE UNITED STATES DISTRICT COURT
FOR THE SOUTHERN DISTRICT OF GEORGIA
SAVANNAH DIVISION

CHRIS JAZAIRI, Plaintiff. VS. ROYAL OAKS APARTMENT ASSOCIATES, L.P., Its Parent Company And Subsidiaries, And MITCHELL L. MORGAN MANAGEMENT, INC., Defendants.	)	CIVIL ACTION FILE NO.: CV-04-404-091
STATE OF SOUTH CAROLINA COUNTY OF CHARLESTON	)	**AFFIDAVIT OF KENNETH WARREN, CIH**

1. Affiant is of legal age and gives this affidavit based on personal and professional knowledge with the understanding that his affidavit will be filed on Plaintiff's behalf in the case of *Chris Jazairi v Morgan Mitchell Properties, et al.*, U.S. District Court, Southern District of Georgia, Savannah Division, Civil Action No. CV-04-404-091.

2. Affiant is an industrial hygienist certified in the Comprehensive practice of Industrial Hygiene by the American Board of Industrial Hygiene and has performed environmental assessments for working and living environments in the usual course of this professional practice. Affiant works for S&ME, Inc., an engineering firm, and is a senior industrial hygienist with S&ME, Inc. He has performed mold assessment projects in the past and has determined if mold remediation is necessary. As part of his work as an industrial hygienist,



Affiant uses various methods of analysis to assess the need for remediation in indoor environments. Affiant's methodology includes the review of available photographs, reports and test data. Affiant often performs a visual inspection and obtains his own testing if he has been retained to assess an indoor environment. In the present case, Affiant is performing a forensic assessment after a remediation has occurred and so the environment is not available for inspection. However, the report of the inspector of the Chatham County Dept of Health (CCDH) was available for review together with photographs, bulk sample test data and statements of the conditions of the apartment.

3. Affiant understands that a toxicologist has criticized his methodology of determining that the level of mold exposure in Royal Oaks Apt. 1607 was probably amplified from the outside air. However, the use of visible mold as an indicator of conditions that could cause adverse health effects to occupants is well accepted in the field of industrial hygiene. Affiant recognizes that the presence of visible mold growth in the occupied indoor environment is strong evidence that mold exposure may occur and that conditions causing mold growth should be corrected and the mold remediated (Bioaerosols: Assessment and Control, 1999, para 19.5.3). The determination of visible mold is the most important assessment tool is noted as an acceptable methodology. Affiant uses the presence and extent of visible mold in his usual practice as an industrial hygienist in his primary determination that mold remediation should be performed, regardless of what types are present and whether air, surface or bulk samples are taken (The Facts About Mold, AIHA, 2003).

4. The use of air tests in determining the extent of mold exposure in an indoor environment is an additional assessment tool but should not always be part of an assessment

because remediation strategies can usually be made on the basis of a visual inspection. In addition, air sampling methods for some mold are prone to false negative results and, therefore, cannot be use to definitively rule out contamination (Guidelines on Assessment and Remediation of Fungi in Indoor Environments, New York City Department of Health, 2001, para. 2.3). Air testing can also be unreliable because the level of mold spores may vary dramatically over time, vary because of seasons and weather, and an airborne exposure limit has not been established for comparison. Significantly different air mold spore levels can exist in a single hour in an indoor environment due to the life cycle and diurnal patterns of molds. (Guidance for Clinicians on the Recognition and Management of Health Effects Related to Mold Exposure and Moisture Indoors, 2004, page 18). Also, air mold spore levels can differ between rooms at the same time due to air distribution patterns caused by the HVAC system.

5. Affiant is not attempting to determine the exact level of mold spores present in the indoor environment based simply on the bulk samples collected by the CCDH and analyzed by AQS Laboratories. However, these test results are part of the data that identifies certain indicator mold types that may signal moisture presence or a potential for health problems to occupants in Royal Oaks Apartment 1607 (Bioaerosols: Assessment and Control, 1999, para 19.5.1.1 and 19.5.1.3) (Guidance for Clinicians on the Recognition and Management of Health Effects related to Mold Exposure and Moisture Indoors, 2004, page 19). This data, along with the factual report of visible mold from the CCDH and the photographs all provide data for consideration in determining that, more likely than not, that occupants in Royal Oaks Apartment 1607 were exposed to mold spore levels at higher levels indoors than outdoors. Affiant would have used this same general methodology in initially assessing the conditions with respect to mold in this

apartment even if the apartment was not the subject of litigation. Affiant has used his professional judgment as a certified industrial hygienist in forming his decision.

6. Affiant also recognizes that porous materials, such as pillows, fabric furniture, curtains, etc., from which mold growth cannot be adequately cleaned should be removed from the building. If porous materials have absorbed odors, removal of the material may be necessary (Bioaerosols: Assessment and Control, 1999, para. 15.5.2). Porous materials that have mold growing on them may have to be discarded, as mold will infiltrate porous materials and complete removal may be impossible. (Mold Remediation in Schools and Commercial Buildings, EPA 402-K-01-001, 2001, page 17).

AND FURTHER AFFIANT SAYETH NOT.

Kenneth R. Warren

KENNETH R. WARREN, CIH

Sworn to and subscribed

before me this 7th day

of March, 2005.

Janet Carwile

Notary Public, 11-04-06

Charleston County, South Carolina

(NOTARIAL SEAL)

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Bioaerosols: Assessment and Control

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FOREWORD

When Theodore Hatch reviewed the advances made in preventing occupational disease in the first half of this century, he observed that progress had been made as a result of interdisciplinary collaboration " . a true melding, even to the point where the different individuals making up the group lost sight of their respective fields and functioned together as a unit, each making his own peculiar contribution, but always as part of the whole" (Hatch, 1964) This book, produced by the ACGIH Bioaerosols Committee in 1998, is a perfect example of the continued progress we can make by following Hatch's guidance In this case, the product is a comprehensive guide to the assessment and control of bioaerosols A glance at the list of editors and chapter authors reveals the remarkable diversity in professional backgrounds and specialties of the contributors. This group has converged upon the need to prevent occupational disease by applying the industrial hygiene paradigm of recognition, evaluation, and control to the set of hazards loosely termed *bioaerosols*. The result is *Bioaerosols Assessment and Control*, which will help the industrial hygienist, indoor environmental specialist, and other occupational health professionals learn from the wide range of people who contributed their expertise to advance the state of knowledge of biologically derived airborne contaminants Hatch would have been proud

There is a small measure of irony in the publication of this book by an organization in a profession which still identifies itself by title as "industrial." The ACGIH Bioaerosols Committee has prepared guidance which will be useful in the full range of workplaces, including those which do not fit the traditional image of "industrial " These indoor workplaces, are in fact the environments in which most people spend their working time The ubiquitous nature of biologically derived contaminants, and their importance as causal agents of work-related disease, are actually among the factors which forced our profession to broaden its scope to the non-industrial workplace As workers in these apparently benign environments began to demonstrate symptoms which they attributed to their workplace, industrial hygienists first applied the tools we had available for evaluating the manufacturing environment In many cases, the measurements of chemicals as gases, vapors, and aerosols revealed exposures far below the levels we had found in the manufacturing world There was a tendency to dismiss the workers' complaints, because the exposures we measured in their environments did not approach the levels we had become accustomed to finding in the workplaces where industrial hygienists had traditionally practiced In retrospect, it is clear that we were wrong to dismiss the workers for at least two reasons. First, we tended to discount evidence of a causal association between symptoms and the workplace because the exposure levels were below the limits This was done despite the admonitions of the TLV Committee and others that the exposure limits are not meant to be interpreted as hard lines between safe and unsafe conditions. Second, we were not sufficiently aware of the limitations of our measurement methods, especially the fact that we usually made no measurements at the biologically derived contaminants which are the subject of this book As the ACGIH guide clearly documents, an investigation of work-related disease must include some assessment of possible exposure to biologically derived contaminants, and this book will put this important tool into the hands of future investigators

Finally, the book illustrates the application of the scientific method to the study of occupational hazards and disease This is remarkable only by comparison to the conventional approach to industrial hygiene, which is frequently compliance-driven But how does one design and conduct an investigation in a setting where there are no relevant exposure limits? The approach the Bioaerosols Committee has presented in this book is founded upon the scientific method of investigating potential causal relationships between exposure and disease It is a thoughtful application of the scientific approach to workplace investigations which can and should be used over the full range of exposures and hazards, not just the biologically derived contaminants which are the subject of this book

So *Bioaerosols Assessment and Control* presents in a single volume a comprehensive guide to the recognition, evaluation, and control of biologically derived contaminants. It is a remarkable product of volunteer effort, and represents a significant step toward the improvement of workplace conditions

Robert F Herrick, Sc D , C.I H

References

Hatch, T Major Accomplishments in Occupational Health in the Past Fifty Years *Ind Hyg J* 25 108-113 (1964)

factor are related parameters that determine if the progression can continue and if the outcome will be a change in health status.

Ideally, documentation that an agent causes a particular disease includes identification of (a) an agent, (b) its immediate, local source and environmental reservoir, and (c) exposure sufficient to cause the response observed. Data may consist of the identification of a source (observational data), measurement of the concentration of a biological agent at the source (bulk sampling data), or measurement of the concentration of the agent in air at a particular time (air sampling data). To establish a clear connection between an exposure and an outcome, an investigator must work through all the steps in Figure 7.1. Unfortunately, data are seldom available on release of biological agents from sources, bioaerosol decay, airway deposition of bioaerosols, agent release to the body from inhaled and deposited particles, or the human factors determining host response. Further, so little is known about these factors for most biological agents that extrapolation from available general information to the specifics of a given case may be uncertain. However, good quality data from environmental investigations can be interpreted as being representative of environmental conditions, and extrapolation of these data to indicate exposure is also often possible, as discussed in the next sections.

7.3.1 Dealing with Visible Microbial Growth

Various authors have recommended that microbial growth in occupied interiors, in HVAC systems, and on building materials and furnishings, especially if extensive, should be avoided and that any contamination that exists should be removed and further contamination should be prevented (Samson et al , 1994; Health Canada, 1995; Maroni et al., 1995; ISIAQ, 1996). "Extensive" visible fungal growth has been defined as surface areas greater than 3 m^2 (32 ft^2) (NYCDH, 1993, Health Canada, 1995; ISIAQ, 1996).

It is well established that bioaerosols cause infectious and hypersensitivity diseases and that bioaerosols in the indoor environment may cause toxic effects, although data on inhalation exposure is limited. Therefore, it is reasonable to use indicators of environmental contamination as a basis for evaluating the need for improved maintenance or remediation in a preventive context. It is also reasonable to use this approach as a means of making decisions in response to outbreaks of BRIs and BRSs. However, caution should be taken in ascribing specific causal links, as described in Chapter 14. At the time of this publication, there is no scientific basis for applying specific exposure limits for concentrations of total or specific culturable or countable bioaerosols [see 1.2].

7.3.2 Comparison with Existing Standards or Guidelines

In the U.S., no federal agency has clear authority to regulate exposure to biological agents associated with BRIs. The OSHA General Duty Clause and Hazard Communication Standard have been used to resolve IEQ problems, for example, to protect remediation workers and building occupants during clean-up operations and to inform building occupants of probable exposure to significant amounts of potentially harmful biological agents (Morey, 1992). The situation may differ in other countries and investigators should be familiar with federal and local regulations relating to bioaerosol exposures.

Workplace exposure limits are based on epidemiological and measurement data (Vincent, 1995). Epidemiological studies examine dose–response relationships and lead to health-based exposure criteria. The dose needed to produce a given response must be related to many factors, for example, the air concentration of an agent, the time period workers are exposed to the agent, the deposition and retention of particles within the respiratory tract, the concentration of the active agent at the target tissue, and the potency of the agent. Measurement-based studies used to establish exposure criteria provide comparative or relative data for problem and control environments. From comparisons of the resulting data, exposures are derived that may be considered acceptable, tolerable, or unlikely to cause harm. If available, data from studies of controlled human or experimental animal exposures may also be considered in the establishment of limit values.

By far, comparison of an environmental measurement with an existing standard is the simplest method to interpret data. Providing data are collected in the manner that

ENVIRONMENT		HOST	
SOURCE → OF AGENT	EXPOSURE → TO AGENT	DOSE → OF AGENT	RESPONSE TO AGENT
Dissemination of agent	Air concentration of agent Time spent in environment	Breathing rate Particle deposition Release of biologically active agent	Host sensitivity/ susceptibility Adequacy of host defenses

FIGURE 7.1. Steps connecting a biological agent and a host response.

7.4.1.4 Significant or Substantial Health Risk Some information is available on concentrations of infectious bacteria that have been associated with disease outbreaks. For example, elevated concentrations of *Legionella* spp. in cooling towers have been linked epidemiologically with disease outbreaks. Likewise, tuberculosis transmission has been related to source strength and ventilation rate. However, little is known about the potential effects for healthy adults and children of exposure to the majority of bacteria recovered during routine air and source sampling. Often, it is only possible to establish that an unusual exposure situation does or does not exist with respect to a control environment.

7.4.2 Fungi

Many fungi produce allergens and some fungi produce toxins. Fungal growth in buildings is undesirable and may cause health problems for building occupants. Although it may be difficult to establish that exposure to fungal aerosols occurs or that exposure presents a hazard, indoor fungal growth is inappropriate and should be removed. Further, steps should be taken to correct conditions that led to fungal growth so that it does not recur. Visible contamination that is confirmed by source sampling to be fungal growth is evidence of indoor contamination. Air sampling (culture or spore-trap sampling) may also indicate indoor fungal growth but should be followed by inspection and source sampling to identify the location of fungal contamination.

In the presence of the inevitable background concentration, the challenge for environmental sampling is to detect indoor fungal growth or entry of fungal aerosols from sources near OAIs and to document the contribution of such sources to occupant exposure. Interpretation of possible indoor fungal exposure has been addressed using (a) indoor/outdoor total concentration ratios, (b) comparisons of the species compositions indoors and out, and (c) the presence of indicator species in the indoor environment.

7.4.2.1 Indoor/Outdoor Comparisons The concentration of fungi in indoor air typically is similar to or lower than the concentration seen outdoors. Exceptions are enclosed agricultural and other specialized environments (where indoor fungal concentrations may be much higher). Outdoor concentrations may exceed those measured indoors even where indoor fungal growth is obvious. If outdoor fungal concentrations are very high, indoor/outdoor concentration ratios for total fungi may be low, even in the presence of significant indoor growth. On the other hand, outdoor fungal concentrations may be reduced during times of snow cover or other conditions that suppress the release of fungal spores from outdoor sources, at which times, indoor measurements may be higher than those outdoors even in the absence of significant indoor sources. Finally, if the variability of the data is high (which is common), extensive sampling may be required to establish that two locations differ. The species of fungi found in indoor and outdoor air typically are similar if outdoor air is the primary source for the fungi in indoor air. Comparisons of the species compositions of indoor and outdoor populations requires accurate identification of fungal species not simply identification to the genus level.

7.4.2.2 Indicator Species Fungi whose presence may indicate excessive moisture or a specific health hazard have been termed indicator organisms. Interpreting the presence or absence of an indicator species requires the ability to identify fungi to the species level and a knowledge of the prevalence of the indicator species in both indoor and outdoor environments. The mere presence of a few CFUs or spores of any fungus should be interpreted with caution. Identification of a particular fungus in an indoor environment does not allow an investigator to conclude that building occupants are being exposed to allergenic or toxic agents. Investigators should also recognize that fungi that have been named indicator species are not the only fungi of significance. Many fungi other than those specifically listed by various groups may cause problems for building occupants exposed through inhalation of aerosols or by other contact.

7.4.2.3 Potentially Pathogenic (Infectious) Fungi Some fungal pathogens should be assumed to be present when materials known to support their growth are found (e.g., *Histoplasma capsulatum* and *Cryptococcus neoformans* in bird and bat droppings). Removal of such materials should be conducted as if they contained pathogenic fungi. Disturbance of soil or other material that may contain fungal pathogens (e.g , compost containing *Aspergillus fumigatus* or material enriched with bird or bat droppings) should be conducted with consideration that occupants of neighboring buildings may be exposed if airborne fungal spores enter the buildings.

7.4.3 Amebae

Investigators should be aware that amebae can cause inhalation fever (e.g., humidifier fever), severe eye and wound infections, and fatal encephalitis, although these conditions are rare. The size of amebic trophozoites and cysts causes them to fall rapidly from air and greatly diminishes the risk of infection when these amebic forms are discharged as aerosols. Physicians should consider the presence of pathogenic amebae when a patient's infection does not respond to traditional antibiotic treatment. Potential environmental sources of amebae should be tested for pathogenic types if infections are identified. These tests should be conducted by laboratories with experience in the assay of pathogenic amebae. Matching

Close consultation with laboratory personnel is vital in planning a bulk sampling program. The type of material to be tested, the biological agents sought, the information needed about the agents, and the expected results determine the appropriate collection method. Laboratory and field staff should discuss how much material is required to conduct particular assays, the number of samples needed to obtain representative results, the number of samples the laboratory can handle per day so that sample processing is not delayed beyond an acceptable holding time, and required sample storage and shipping conditions [see Chapter 6 and Part III]. Samples to be tested for viable microorganism counts generally require overnight delivery and need to be either chilled (i.e., maintained between ~4° and 10°C) or kept at room temperature but protected from extremely high or low temperatures during transport. In some cases, preservatives or an agent to neutralize a biocide (e.g., sodium thiosulfate for chlorine in water samples) are added in the field to stabilize samples and limit changes prior to analysis (Thorne et al., 1994; APHA, 1995). Culture plates can also be inoculated at the collection site prior to sample shipment, and convenient dipslides are available for some types of water testing (Biotest Hycon, Denville, NJ; Difco Laboratories, Detroit, MI).

12.1.3 Sample Analysis

Chapter 6 and the individual chapters in Part III describe methods for analyzing biological agents in bulk samples. These methods include detection of culturable microorganisms and morphologically distinctive particles as well as bioassays and chemical assays (AIHA, 1996). Dust samples are often sieved to collect the finer particles, which are those most likely to have been or to become airborne. Biological agents in weighed portions of collected dust and bulk materials are typically suspended or extracted in a fluid appropriate for the assay to follow. Sections 13.2.2 and 13.2.3 describe the calculation of the concentration of biological agents in bulk samples. Occasionally, bulk samples are collected for archival purposes and are not analyzed immediately. Unused portions of bulk samples may also be stored for later retesting. Storage conditions must minimize changes in the biological agents of interest. In particular, assays for many living organisms are of limited value after prolonged sample storage.

12.1.3.1 Culture-Based Analyses Culture-based analyses involve growing microorganisms from bulk samples. For example, sieved dust as well as liquid samples, dust suspensions, and washings of other materials may be inoculated onto suitable agar-based culture media or cell cultures (Samson et al., 1994, AIHA, 1996). The type of growth medium or cells used and the incubation conditions (e.g., temperature, humidity, atmosphere, and duration) influence what bacteria, fungi, viruses, or amebae can be isolated. In addition, factors related to the sampled environment, the microbial agents, and the methods of sample collection and handling determine how representative the culture results are of the types and relative proportions of microorganisms present in bulk samples.

12.1.3.2 Non-Culture Analyses Non-culture analyses for bulk samples may involve identifying biological agents under a microscope or employing biological or chemical assays. The information available from these analyses varies with the biological material under study. Non-culture methods generally do not provide information on the ability of identified microorganisms to propagate or to cause infection. Many fungal spores and pollen grains as well as dust mites and some amebic cysts can be identified by direct microscopic examination because of the organisms' distinctive sizes, shapes, and surface features. However, bacterial cells and viruses are generally much smaller and less distinctive. Few bacteria can be identified by microscope without some sort of staining, and viruses are only visible with an electron microscope. In some cases, bulk samples are analyzed to detect the presence of antigens from fungi, dust mites, cockroaches, birds, or mammals. Bulk samples can also be tested for the presence of microbial products and marker compounds (e.g., endotoxin, muramic acid, ergosterols, VOCs, and specific nucleic acid sequences)

12.1.3.3 Information from Bulk Samples Some detection methods provide only semiquantitative information and are used only to identify the presence or absence of the biological agent in question. However, even such limited information may help investigators decide if further sampling is indicated and may help them formulate recommendations for remediation.

Bulk samples can also provide material with which to immunologically test exposed workers. Positive reactions only in symptomatic workers or the occupants of a problem area could reflect exposure to environmental antigens even though the specific antigenic material was not identified. Similarly, extracts of microorganisms isolated from environmental samples by culture-based methods can be used to determine workers' immunological sensitivity to specific bacteria or fungi. Tests conducted with extracts of dust samples or microorganisms isolated from an actual workplace may have more value than tests run with a standard extract battery such as an HP panel [see 3.3.3]. The antigen mixtures available in commercial panels may not include the relevant biological agents, or the microorganisms in a work environment may differ from those used to make commercial extracts even if they are identified as the same genus and species.

The concentration of a biological agent in a bulk sample is determined from the number of cells, number of CFU, or amount of target material per area sampled or per mass or volume of material analyzed. For example, bulk

sampling results could be expressed as the number of fungal spores per square centimeter of ceiling tile, number of bacterial CFU per gram of insulation material, or nanograms of endotoxin per milliliter of cooling-tower water. Numerical measurements can help investigators compare locations within a workplace and relate current findings with those from other studies.

12.1.4 Interpreting Sampling Results

Investigators need experience to interpret bulk sampling data. In some cases, it may be sufficient to demonstrate that a specific biological agent was found in a study area. However, failure to detect a target material does not necessarily mean it is not present. Laboratories should report such results as less than the test's LDL rather than as zero [see 5.2.3 and Chapter 7]. Because bulk samples are not good estimators of actual exposure, measurements of biological agents in bulk samples do not allow conclusions as to the air concentration of material that would be associated with a given disease outcome. However, measurements of inhalation exposure may be difficult for some biological agents (e.g., some antigens), and positive correlations have been seen between health effects and some antigen concentrations in dust samples [see 22.5 and 25 5].

12.1.4.1 Differentiating In-Situ Microbial Growth from an Accumulation of Material of Biological Origin On microscopic examination or laboratory culture, the finding of predominantly one type of microorganism or the presence of hyphae and spore-bearing fungal structures (rather than a mixture of fragmented hyphae and spores) may indicate that growth is occurring at the sampled site rather than that biological material has simply collected there. However, water in drain pans, cooling tower sumps, or humidifiers is not expected to be sterile (i.e., some microorganisms are likely to be present even if not actively multiplying).

12.1.4.2 Basing Recommendations on Bulk Sampling Data Detecting high concentrations of microorganisms in bulk samples may eliminate the need for air sampling if there is a clear potential for the material to become aerosolized or for building occupants to directly contact the contaminated items. Recommendations for remediation might also be straight forward because such a situation is generally undesirable and should not be allowed to persist [see 14.1 2] For example, investigators who find biofilm or large areas of visible fungal growth in an air handler should suggest mitigation of the problem rather than further sampling. However, air sampling is needed to establish worker inhalation exposure in terms of amount or type of biological material [see 14 1 3].

Part of the difficulty in determining that material is biologically contaminated and in establishing a connection between bulk sample measurements and health complaints stems from the many factors that can affect sampling results. Large variability has been observed among sampling sites in buildings and even at a single sampling location over time, reflecting seasonal effects and changing environmental conditions. Significant biological agents may be missed, misleading investigators, if the choice of collection and analytical methods were inappropriate or insufficiently sensitive. Therefore, investigators must consider bulk sampling data in conjunction with all available medical and environmental information to decide if the data support the hypotheses under evaluation Further, investigators must decide what evidence is sufficient to warrant recommendations for remediation of suspected sources of biological agents. Accurate determination of the type and extent of indoor biological contamination is critical in selecting suitable remediation methods and appropriate precautions during the cleanup process [see Chapter 15].

12.2 Surface Sampling

12.2.1 General Considerations

Surface sampling during IEQ investigations is frequently linked to bulk and air sampling. Surface samples can provide information similar to that obtained from bulk samples regarding whether environmental materials may be contaminated beyond background levels and possibly serve as sources of biological agents that may be disseminated as bioaerosols. In addition, building occupants may be exposed to biological agents via skin contact with contaminated indoor surfaces.

Surface sampling may be used to (a) confirm the nature of suspected microbial growth on environmental surfaces, (b) measure the relative degree of biological contamination, and (c) identify the types of microorganisms and other biological agents present. Surface sampling is preferred over bulk sampling when a less destructive method of sample collection is desired. For example, it may be possible to collect samples of fungal growth from the surfaces of valuable furnishings or materials of historical interest without damaging the original items

The concentration and composition of microorganisms growing on indoor surfaces depend on a number of factors, such as the nature of the material and its moisture history. Surfaces may also become contaminated as a consequence of bioaerosol deposition, for which the relevant parameters are the size, shape, and density of the biological particles; air velocities past surfaces; and air movement in the area. Researchers have collected gravity samples by exposing glass slides or settling plates in sample areas to collect whatever particles fall onto a surface in a given time (Pasanen et al., 1992) However, comparisons of data from gravity or sedimentation samples and active air samples rarely agree in their assessments of either bioaerosol concentration or composition (Hyvarinen et al., 1993, Ren et al., 1993). While gravity samples may reflect surface deposition during a sampling period, they are not suitable substitutes for volumetric air samples [see 11 3].

12.2.2.2 Surface-Wash Sampling In the surface-wash method, a swab, filter, or cheesecloth or gauze swatch is used to wipe a specified surface area (Reynolds et al., 1990, Sandholm and Wirtanen, 1993; Morey, 1994). The collection media may be wetted with sterile water or wash solution (e.g., 0.1% peptone water with 0.01% Tween 80) to enhance particle collection. Samples for culture-based analysis must be handled aseptically; for example, by using sterile forceps or touching only the bare end of a swab stick. A swab can be used to inoculate a culture plate immediately, or swabs, filters, and swatches can be shipped to a laboratory for analysis. Samples may be transferred to a laboratory dry in individual sterile containers or in a test tube with a sterile transport medium. Wipe samples can be processed similarly to dust samples (e.g., mechanically agitated in a sterile wash solution followed by culture-plate or cell-culture inoculation or other appropriate assay).

12.2.3 Sample Analysis

Chapter 6 and the individual chapters in Part III describe methods to analyze biological agents in surface samples. These methods include detection of culturable microorganisms and morphologically distinctive particles as well as bioassays and chemical assays (AIHA, 1996). The concentration of biological material in surface samples is expressed in terms of CFU, particles, or other unit of measurement per area sampled. Sections 13.2.2 and 13.2.3 describe the calculation of the concentration of biological agents in surface samples.

12.2.3.1 Culture-Based Analyses Culture-based analyses involve growing microorganisms from surface samples. For example, contact plates can be incubated directly, and swab samples and other surface washings can be inoculated onto agar-based culture media or cell cultures. The type of growth medium or cells used and the incubation conditions (e.g., temperature, humidity, atmosphere, and duration) influence what bacteria, fungi, viruses, or amebae can be isolated. In addition, factors related to the sampled environment, the microbial agents, and the methods of sample collection and handling determine how representative culture results are of the types and relative proportions of microorganisms present on surfaces.

12.2.3.2 Non-Culture Analyses The primary non-culture analysis performed on surface samples is examination of collected material by microscope [see 12.1.3.2].

12.2.4 Interpreting Sampling Results

Investigators need experience to interpret surface sampling results. The information expected from contact surface samples is often simple confirmation that the collected material is biological in nature or that biological growth can be ruled out. Failure to detect a target material does not necessarily mean it was not present, but simply that the collection and analytical methods did not detect it. The reliability and relevance of information obtained from culture-based surface samples depends on sample number, where and how samples were collected, and the overall design and purpose of the study. For example, the concentration density of biological agents on the ceilings, walls, and floors of rooms and ventilation ducts may differ substantially. Morey (1994) found significant variations in fungal concentrations in a single diffuser, which suggests that a large number of samples is needed to accurately characterize an area by using surface sampling. Estimating the total surface area assumed to be similar to each sampled section is important because different levels of precaution have been recommended for remediating different degrees of contamination with potentially toxigenic fungi [see 15.2].

The recovery efficiency of various surface sampling methods differ, and some biological materials may be more difficult than others to remove from surfaces. For example, Sandholm and Wirtanen (1993) found that microorganism removal from even relatively smooth surfaces could be difficult and that release of cells to the environment could occur in a random fashion.

Porous or fleecy surfaces (e.g., duct insulation, upholstered furniture, and carpets) are even more difficult to sample adequately than smooth surfaces (e.g , wood, tile, or vinyl floors; table and desk tops; and metal surfaces). Debris other than microbial fragments and spores is sometimes so abundant on environmental surfaces that it obscures accurate recognition and identification of microbial contaminants. This may be especially problematic when collecting samples from fabrics or friable surfaces such as plaster or concrete. Investigators are advised to take multiple samples to improve the chances that at least one will be satisfactory. They also may collect more than one sample from the same area on the chance that the first will remove the grosser particles, leaving the finer material to be removed subsequently.

Several attempts have been made to identify surface concentrations of biological materials that indicate unhealthy conditions. No currently available guidelines have been generally accepted due to the large variability in surface sampling results and poor correlations with inhalation exposure. In general, immediate mitigation is needed for microbial growth found on materials that are in direct contact with indoor air or subject to disturbance that might release biological particles, as well as for materials that building occupants may contact directly [see 14 1.2 and 14.1 3]. However, information on cause–effect relationships between surface concentrations of biological materials and illness is not currently available. Therefore, investigators must consider surface sampling data in conjunction with all available medical and environmental information to decide if the data support the hypotheses under evaluation and if the evidence warrants a recommendation for source removal, cleaning, or repair.

Chapter 15

Remediation Of Microbial Contamination

Richard J. Shaughnessy and Philip R. Morey

15.1 Introduction

Prevention of microbial growth indoors is only possible if the factors that may allow it are identified and controlled. When prevention has failed and visible microbial growth has occurred in a building, restoration requires (a) removal of porous materials showing extensive microbial growth, (b) physical removal of surface microbial growth on non-porous materials to typical background levels, and (c) reduction of moisture to levels that do not support microbial growth. Preventing water intrusion during the remediation process is advised to prevent further microbial growth. However, Foarde et al. (1997) found that lowering indoor RH triggered spore release from fungal contamination on duct material. Therefore, remediators should consider whether contaminated materials should be removed before measures to thoroughly dry the environment are undertaken. Identification of the conditions that contributed to microbial proliferation in a building is the most important step in remediation. No effective control strategy can be implemented without a clear understanding of the events or building dynamics responsible for microbial growth [see 10 1].

15.2 Removing Existing Contamination

Growth that has occurred in a surface layer of condensation on painted walls or non-porous surfaces (including wood) can usually be removed by (a) vacuuming using equipment with high-efficiency filters or direct air exhaust to the outdoors, (b) washing with a dilute solution of biocide and detergent, or (c) cleaning, thorough drying, and repainting. Porous materials that have sustained extensive microbial growth must often be removed. Examples of porous materials are ceiling tiles, installed carpeting, upholstered furnishings, and wallboard. Extensive microbial growth refers not only to the extent of the area affected but also the degree to which microorganisms have degraded a material for use as a food source. "Extensive" visible fungal growth has been defined as surface areas greater than 3 m^2 (32 ft^2) (NYCDH, 1993, Health Canada, 1995, ISIAQ, 1996) Carpeting and drapes that can be removed for thorough cleaning and drying may be salvageable. Valuable books and papers can sometimes be rescued by fumigation, followed by freeze-drying and vacuum removal of residual particles.

The removal and cleaning of contaminated materials must not be undertaken without proper precautions, because disturbance of contaminated materials can result in bioaerosol release (Flannigan, 1992). Disturbance of microbial growth in air-handling systems may lead to the dissemination of bioaerosols throughout a building (Morey and Williams, 1991). Concentrations of airborne spores indoors during material disturbance and removal may approach levels characteristic of dusty agricultural environments (Hunter et al., 1988; Rautiala et al , 1996). Resulting exposures to biological agents may compromise the health of remediation workers and building occupants.

When visible contamination is extensive, containment procedures similar to those used to handle hazardous wastes (e.g., asbestos) are required to safely remove contaminated materials (Morey, 1994) Remediators can consider using the recommendations others have developed to handle removal of materials visibly contaminated with potentially toxigenic fungi (NYCDH, 1993; Morey, 1992, 1994, 1996; Health Canada, 1995; Morey and Ansari, 1996) Recommended removal methods take into consideration both the nature and extent of contamination, that is, the particular microorganisms present and the amount of material or area affected. Such work should be conducted while buildings are unoccupied (NYCDH, 1993, Weber and Martinez, 1996) Investigators should decide if uncontaminated items in an area to be remediated should be removed to protect them during the cleanup process or if covering the items will provide enough protection

With appropriate PPE, local maintenance personnel should be able to remediate visibly contaminated areas of less than 3 m^2 (NYCDH, 1993). However, many factors must be considered in deciding what level of precaution is appropriate and how contaminated materials should be contained and removed (IICRC, 1995; ISIAQ, 1996, Morey and Ansari, 1996). In general, the removal and containment precautions required for toxigenic fungi should be used for remediating any visible fungal contamination because virtually all fungi can cause allergy (in sensitized individuals) and many fungi produce toxins (Morey, 1996, NYCDH, 1993) (Table 15.1).

15.2.1 *Source Containment*

Table 15.1 refers to three containment requirements for varying degrees of contaminant removal. Source containment may be as simple as placing a moldy ceiling tile

TABLE 15.1. Guidance for Removing Visible Fungal Growth

CAVEAT This table is presented as a general guide only Factors besides those listed may need to be considered when deciding what levels of environmental and personal protection are appropriate during remediation activities (e g , the contaminating agent, the nature of the contaminated material, and the location of the site requiring remediation). Categorizing the extent of contamination requires professional judgement.

Visible Fungal Growth[A]	Recommendations to Prevent Dust or Spore Dispersion[B]	Suggested Minimal PPE
Minimal	*Source containment:* material removal with minimum dispersal of dust and spores	N-95 respirator, gloves
Moderate	Local containment: enclosure and negative pressurization to prevent dispersion of dust and spores	N-95 respirator;[B] eye protection, full-body covering[C]
Extensive	Full containment· critical barriers and negative pressurization to contain dust and spores; personnel trained to handle hazardous wastes	As above

(adapted from NYCDH, 1993; Morey, 1992, 1994, 1996, Health Canada, 1995, Morey and Ansari, 1996)

[A] "Visible contamination" means that fungi are readily observable on surfaces. The presence of hyphae and mycelia on or in materials, as seen by direct microscopic examination, verifies that visible contamination is of fungal origin. [Categorization of the extent of microbial contamination and determination of the required level of containment and appropriate PPE involve professional judgement.]

[B] Higher levels of respiratory protection [e.g., half- or full-face respirators or full-face powered air purifying respirators (PAPRs) with HEPA cartridges] may be considered necessary for some remediation work. Investigators should seek the opinions of occupational physicians, toxicologists, respiratory protection experts, or health and safety professionals to select appropriate PPE.

[C] "Full-body covering" is defined as the collective use of full-body disposable coveralls, head covering, eye protection, gloves, and shoe covers

19.2.2.2 Hypersensitivity Pneumonitis HP may also result from exposure to fungal antigens. For HP development, it appears that heavy, continuous or repeated exposure to small fungal particles is essential. Genetic factors that may control susceptibility are unknown [see also 3 3.3 and 8 2 1.3]

19.2.3 Toxic Effects

19.2.3.1 Mycotoxins Mycotoxins produce a variety of health effects via ingestion, skin contact, and inhalation [see 24 2]. Depending on the kind of mycotoxin and the nature of the exposure, effects may include mucous membrane irritation, skin rashes, dizziness, nausea, immunosuppression, birth defects, and cancer. Nearly all of the mycotoxin literature focuses on ingestion exposure, although the role of inhaled mycotoxins in human disease is currently under scrutiny. In view of the potential severity of resulting diseases, a conservative approach to limiting exposure to mycotoxins is recommended [see 24.6].

19.2.3.2 Glucans Glucans comprise the bulk of the cell walls of most fungi. Glucans have antitumor activity and modulate the endotoxin-stimulated release of cytokines in Gram-negative bacterial infections (Williams et al., 1996). Glucans have irritant effects similar to—although less potent than—those of endotoxin. Exposure to glucans in dust has been associated with BRSs (Rylander et al , 1992; Rylander, 1995). Whether the glucans, some other fungal agent, or other factors associated with conditions leading to fungal growth actually mediated the effects remains to be investigated [see 24 7.2].

19.2.4 Volatile Organic Compounds

Fungi produce VOCs while growing and degrading substrates [see 19 1 4]. Some of these compounds have distinctive odors and low odor thresholds, and many people find these VOCs offensive or annoying [see 26.2.2 1]. Exposure to such compounds may be responsible for some nonspecific BRSs. However, the role of fungal VOCs in clinically evident disease has not been studied [see 26.3].

19.2.5 Beneficial Fungal Products

Fungi are well-known as important food sources (e.g., mushrooms, truffles, morels, tempeh, soy sauce, yeast bread, cheese, wine, and beer). In addition, many of our most effective antibiotics (e.g., penicillin, griseofulvin, and cephalosporin) are produced by common fungi. Cyclosporin A, an immunosuppressive mycotoxin produced by the fungus *Tolypocladium inflatum*, is one of the drugs that makes organ transplants possible. In addition, many anti-cancer agents are products of fungal metabolism. As mentioned in Section 19.2.3.2, fungal glucans show promise as agents to minimize the effects of Gram-negative bacterial infections.

19.3 Sample Collection

19.3.1 Sampling Strategies

Visible fungal growth on interior surfaces is clear evidence that fungi have colonized an environment. Although the health impacts of surface growth have not been documented, the potential exists for exposure to fungal allergens, toxic metabolic products, and malodorous VOCs. In most cases, the discovery of visible fungal growth warrants a recommendation for cleanup and identification of the underlying reason for the growth [see 10 1 and 14 1.2]. In many cases, further air or source sampling is not necessary. However, investigators may decide to collect air or source samples to document that fungal growth has occurred or to record the kinds of fungi that predominate. The most successful sampling strategies are those formulated to answer specific questions.

19.3.2 Source Sampling

Investigators who suspect exposure to specific allergens or toxins may collect source samples to evaluate the possible contributions of visible fungi or fungi in dust to specific disease processes [see Chapter 12]. Source sampling is also useful to document that discoloration or deposits on surfaces actually represent either fungal growth or spore accumulation. Simple tape sampling, with microscopic analysis, will often confirm the presence of hyphae and spores as well as allow identification of many kinds of fungi. However, culture of surface or bulk samples may be necessary to allow microscopic identification of some fungi (e.g., species of *Aspergillus* or *Penicillium*) [see 19 4].

Bulk dust samples are often cultured to evaluate the kinds of fungi present In addition, chemical analyses (e.g , measures of ergosterol or glucans) may be used to estimate total fungal biomass. Immunoassays for specific fungal allergens are also under development. Investigations occasionally require collection of bird, bat, or rodent droppings; other animal debris; or soil samples for detection of fungal saprobes that cause human infections (Levitz, 1991, Lenhart, 1994, APHA, 1995, ASM, 1995; Lenhart et al., 1997) [see 19 2.1].

19.3.3 Air Sampling

Air sampling for particles of fungal origin is complicated by their diversity in size, shape, density, and surface features—all of which affect particle behavior while airborne and during collection. Most commonly, airborne fungal spores are collected using impaction onto agar or an adhesive-coated transparent surface (spore trapping) (Samplers 1 to 7 and 10 and Samplers 16 to 20 in Table 11.1). Culture-based analysis tends to underestimate actual fungal concentrations because many spores are either not living or are unable to grow on the culture medium provided [see 19.4]. Spore trapping allows accurate counting of total fungal spores and identification of some spores, but many

spores cannot be identified by microscope (e.g., *Penicillium* and *Aspergillus* spp.).

19.3.4 Sample Handling

Samples of living fungi must be handled carefully to preserve viability if cultural analysis is to be used and to prevent growth before the samples reach a laboratory. For microscopy or chemical analysis, it may be possible to treat samples with a biocide or dry them to kill the cells or prevent replication. Stored properly, treated samples can be held indefinitely. Air and dust samples for immunoassay should also be kept dry, but must be analyzed within approximately 48 hours or frozen if longer delays are necessary. Investigators should consult the mycologist who will examine the samples about how to ship them (e.g., the type of container to use and whether to refrigerate samples or keep them at room temperature). Samples to be cultured should reach the laboratory within 24 hours of collection and should be examined and processed on the day received to minimize changes in the kinds and concentrations of fungi or fungal products present.

19.4 Sample Analysis

The primary methods of environmental sample analysis for fungi remain (a) isolation of fungi by laboratory culture, and (b) microscopic examination of fungal cultures and individual fungal spores. However, other approaches are also used (Table 19.4) (Madelin and Madelin, 1995; AIHA, 1996a,b; Buttner et al., 1997). In particular, analysis of bulk samples for ergosterol or glucan concentration as estimates of total fungal biomass are of increasing interest [see 19.1.4 and 19.2.3.2]. Immunoassays are under development for measurement of some specific fungal allergens. Section 6.3.1 briefly describes how laboratories process samples and how concentrations of culturable fungi are calculated for air and source samples. Table 19.4 compares available methods for identifying common fungi.

19.4.1 Culture

Total (non-differential) fungal colony counts can provide investigators some information for evaluating the microbial status of an indoor or outdoor environment. However, it is essential that fungi be identified if investigators wish to compare bulk, surface, or air samples from different sampling locations or times and to interpret the potential health risks of the fungi present. A well-trained environmental mycologist may be able to identify many fungi to the genus level directly on a culture plate using a low-power dissecting microscope. Characteristics used for identification include colony color, size, and texture; the type of spore-bearing structures present; and, in some cases, the arrangement of the spores on these structures. A few fungi are so distinctive they can be identified to group or even species level by this simple method. For example, a good environmental mycologist can readily assign many fungi to the species level (e.g., *Epicoccum nigrum, Paecilomyces variotii, Paecilomyces lilacinus, Stachybotrys chartarum, Cladosporium sphaerospermum, Pithomyces chartarum, Trichoderma viride, Botrytis cinerea,* and several *Penicillium* spp.). Some *Aspergillus* isolates can also be identified to the species level, and others to the group level, with further characterization pursued where indicated. For identification, fungi must be grown under carefully controlled conditions. Identification of fungal species generally requires skillful use of a high-power, light microscope and highly specific training and experience. Such experience is available from mycologists working in academic research laboratories and others who have obtained specialized training.

Yeasts are often abundant in dust samples but are usually reported only as total colony counts and are seldom identified to genus or species level. Unlike other fungi, in culture, yeasts produce bacteria-like colonies with few distinguishing features. Physiological and biochemical tests are required for yeast identification. Consequently, little is known about which yeasts are common in indoor environments and the potential health effects of aerosol exposure to them. One exception is *Sporobolomyces* sp., which has been associated with hypersensitivity disease and is readily recognized by its distinctive colony morphology and by its production of forcibly discharged ballistoconidia.

19.4.1.1 Culture Media If investigators wish to compare their sampling results with an existing database, they should adopt the same culture medium used in the studies in the database. Except when specific fungi are of special concern, culture media for fungi from air or source samples should support a wide variety of common taxa. Various formulations of malt extract agar (MEA) have a long history of use for air monitoring (Samson et al., 1994, AIHA, 1996a). The second formulation in Table 19.5 is one also used as a diagnostic medium to identify *Aspergillus* spp. This formulation works well in most non-industrial indoor environments, provided rapid-growing fungi, such as *Rhizopus, Mucor, Monilia,* or *Trichoderma* spp., are not abundant. If these fungi are present in large numbers, 2% MEA (without glucose or peptone) or a medium such as DG-18 that contains growth inhibitors can be used.

The addition to fungal culture medium of compounds to suppress bacterial growth is generally needed only for samples from areas where very high concentrations of bacteria ($>10^5$ CFU/m^3) are expected (e.g., agricultural, metal-working, or domestic waste-handling environments). Commonly used bactericides are rose bengal and antibiotics (e.g., chloramphenicol, penicillin, and streptomycin). Rose bengal becomes fungicidal when exposed to light and must be kept covered as much as possible during sample collection and in the laboratory.

Cellulose agar has been used to isolate *S. chartarum*. This fungus grows well on other media (e.g., MEAs, after

initial isolation), but its growth is slow and other fungi in environmental samples may outcompete it. However, few fungi other than *S. chartarum* are able to grow on water agar (20 g agar per L of water) overlaid with sterile filter paper. Therefore, this or other formulations in which cellulose is the only carbon source (AIHA, 1996a) may be useful to isolate this fungus from environmental samples.

19.4.1.2 Incubation Conditions Culture plates are incubated at room temperature (18° to 22°C) for isolation of most fungi. If *only* thermotolerant or thermophilic fungi are of concern, plates can be incubated at temperatures up to 45°C. Some near-ultraviolet exposure is necessary to induce sporulation in many dark-spored fungi and can be provided by sunlight or by placing lamps designed for illuminating house plants in an incubator. Fungal cultures should be monitored daily, but typically colonies cannot be accurately counted or identified before at least five days of incubation. Plates should be disturbed as little as possible during incubation because spores are readily dislodged, and dislodged spores may form new colonies, leading to inaccurate counts.

19.4.2 Microscopy

Mycologists use dissecting and light microscopes to examine fungi from air and source samples. Both species identification of fungi in culture and examination of spore-trap air samples should be performed by experienced mycologists familiar with environmental fungi.

19.4.2.1 Examination of Fungal Structures Many common fungi can be identified to genus and sometimes to species based on macroscopic colony morphology (e.g., color and texture) or spore-bearing structures viewed at 10- to 60x magnification (Table 19.2). More exact identifications can be made by examining stained wet mounts. Fungi that have not sporulated in culture generally cannot be identified. In some cases, these colonies are sterile forms of common fungi, such as species of *Cladosporium*. Although an experienced mycologist can provisionally classify these fungi based on colony morphology, such identification is never entirely reliable. However, most non-sporulating fungi seen in the laboratory are types that are unable to produce spores in culture. Many of these colonies represent growth from sexual spores (ascospores and basidiospores) of fungi that do not produce asexual stages.

19.4.2.2 Spore-Trap Air Samples Many kinds of individual fungal spores are identifiable—at least to general category—when examined at 1000X magnification. Experienced aerobiologists can identify some fungal genera and species from microscopic examination of single spores (e g., *E. nigrum* and *S. chartarum*). In other cases, the genus can be identified from a spore, but not the species (e.g., *Alternaria* and *Cladosporium* spp.). The spores of some genera are similar (e.g., *Aspergillus* and *Penicillium* spp.), and culture is needed to identify these fungi to the species level.

19.4.3 Chemical Analyses

Although culture and microscopy are still the most commonly used methods for analyzing samples for fungal content, chemical analyses for indicators of fungal presence are of increasing interest (e.g., assays for ergosterol and glucans) (AIHA, 1996b, Flannigan, 1997; Saraf et al , 1997) [see 6 7 and 24 7 3]. Requirements for air sample collection for these assay methods differ from the requirements for either culture or microscopy in needing much larger air volumes to overcome the methods' relatively low sensitivities. These methods may prove most useful in highly contaminated environments, such as agricultural settings or wastewater treatment plants.

TABLE 19.4. Relative Usefulness of Available Methods for Analysis of Fungal Samples

Fungal Category	Microscopy	Culture	Ergosterol	Glucan	Immunoassay
Total fungi	++[A]	+	++[B]	++[B]	–
Alternaria spp.	+++	+	–	–	++
Cladosporium spp.	+++	++	–	–	(++)
Stachybotrys chartarum	+++	+	–	–	–
Penicillium and *Aspergillus* spp.	++	++	–	–	–
Penicillium viridicatum	–	++	–	–	–
Aspergillus fumigatus	–	++	–	–	(++)
Aspergillus niger	++	++	–	–	–
Chaetomium globosum	+++	+	–	–	–
Basidiospores	+++	–	–	–	–

[A] most useful for air samples
[B] most useful for bulk samples

– not useful
+ somewhat useful
++ useful
+++ very useful

19.4.4 Immunoassays

Immunoassays are available for detecting a few fungal allergens (e.g., mixtures of allergens from *Alternaria alternata*, *A. fumigatus*, and *C. herbarum*) (Horner et al., 1995) and for a few mycotoxins (e.g., aflatoxin and T-2 toxin) [see 24.4.2 and 25.4]. None of these assays has yet been used extensively in air quality investigations. Large-volume air samples are required for immunoassays to overcome the methods' relatively low sensitivity.

19.5 Data Interpretation

Data from appropriately designed sampling studies should allow investigators to evaluate hypotheses regarding possible exposure to fungal aerosols at a study site. Few health-based guidelines or standards are available to assist investigators in the interpretation of fungal aerosol data, and only limited exposure–dose or dose–response data are available on which to base guidelines. Therefore, the interpretation of data on fungi in air and source samples generally focuses on the fungal genera and species identified, comparisons among different environments, and the potential susceptibilities of exposed populations to various fungal agents.

19.5.1 Other Recommendations

Various groups have offered guidance on the interpretation of environmental air and source samples for fungi. These recommendations are meant to be applied by properly trained and experienced persons who understand the strengths and limitations of the available methods for environmental sampling for fungi.

19.5.1.1 Health Implications of Fungi in Indoor Environments A 1992 workshop in the Netherlands led to the recommendation that certain fungi should be considered indicator organisms that may signal moisture presence or a potential for health problems if above a baseline level (to be established) in air or surface samples (Samson et al., 1994). The following fungi were named as indicator species (an asterisk indicates those fungi the authors considered to be important toxigenic taxa): (a) materials with a_w >0.90 to 0.95: *A. fumigatus* and species of *Trichoderma*, *Exophiala*, *Stachybotrys**, *Phialophora*, *Fusarium**, *Ulocladium*, and yeasts (*Rhodotorula*), (b) materials with a_w 0.85 to 0.90: *A. versicolor**, and (c) materials with $a_w \leq 0.85$: *A versicolor**, species of *Eurotium* and *Wallemia*, and species of *Penicillium* (e.g., *Penicillium chrysogenum* and *Penicillium aurantiogriseum*).

19.5.1.2 Canadian Recommendations Health Canada (1993, 1995) has recommended the following guidelines for recognizing and managing fungal contamination in public buildings. Bird or bat droppings may contain pathogenic fungi (e.g., *C. neoformans* and *Histoplasma* spp.) and toxigenic *A. fumigatus*. Appropriate action (described in the document) should be taken for the safe removal of materials that may contain these fungi. The persistent presence, demonstrated on repeated sampling, of toxigenic

TABLE 19.5. Culture Media for Fungi (ingredients per liter of distilled water)

Category	Medium	Ingredients	
Most saprobic fungi[A]	2% MEA	20 g malt extract 20 g agar	
Most saprobic fungi[B]	MEA	20 g malt extract 20 g dextrose 1 g peptone 15 g agar	final pH 4.5-5.0
Xerotolerant fungi (including fungi cultured from settled dust samples)	DG-18 a_w = 0.955	10 g glucose 5 g peptone 1 g KH_2PO_4 0.5 g $MgSO_4 \cdot 7H_2O$ 0.1 g chloramphenicol 15 g agar 220 g glycerol 2 mg dichloran	final pH 5.6
Antibacterial medium for fungi	MEA with:[C] chloramphenicol rose bengal, penicillin, or streptomycin	0.1 g/L 35 mg/L 20 units/mL 40 units/mL	final pH 5.6

[A] environments in which rapid-growing fungi are abundant

[B] environments in which rapid-growing fungi are not abundant

[C] Except for chloramphenicol, add anti-bacterial agent after autoclaving

fungi (e.g., *S. chartarum* and species of *Aspergillus, Penicillium,* and *Fusarium*) indicates that further investigation and appropriate action (described in the document) should be taken. The confirmed presence of one or more fungal species seen as a significant percentage of an indoor sample but not similarly present in concurrent outdoor samples is considered evidence of a fungal amplifier, and appropriate action (described in the document) should be taken. Fungi in indoor air should be qualitatively similar to and quantitatively lower than what is found in outdoor air, but factors such as sampling technique, season, and weather affect what fungi are isolated from outdoor air. Numeric criteria are suggested for single fungal species (other than *Cladosporium* or *Alternaria* spp.), mixed species reflective of those typically found in outdoor air, and fungi primarily from plants in summer.

19.5.1.3 American Industrial Hygiene Association The AIHA (1996a,b) has offered guidelines for interpreting culture results for air and source samples for fungi. These guidelines state that genera such as *Cladosporium, Alternaria,* and *Epicoccum* as well as Basidiomycetes are present in outdoor air on a seasonal basis. However, in mechanically ventilated buildings with air filtration, the concentrations of these typically outdoor fungi should be lower than concentrations measured at the OAI. Dominance in indoor air of fungal species not predominant in outdoor air indicates that these fungi are growing in a building and that the air quality is degraded. The confirmed presence of *S. chartarum, A. versicolor, A. flavus, A. fumigatus,* or *Fusarium moniliforme* requires that urgent risk management decisions be made (references provided) "Confirmed presence" is defined as colonies in several samples, many colonies in any sample, or, where a single colony was found in a single sample, evidence of the growth of these fungi on building materials by visual inspection or source sampling. The AIHA guide states that certain pathogenic fungi may be problems if present in indoor air (e.g., *A. fumigatus, A. flavus,* and other species that may cause aspergillosis as well as *F. moniliforme, H. capsulatum,* and *C. neoformans*). Because the latter two fungi are difficult to culture and are seldom detectable in air samples, a default assumption is that they may be present in any bird or bat droppings and that disinfection and removal of such material is required.

19.5.1.4 International Society of Indoor Air Quality and Climate The International Society of Indoor Air Quality and Climate (ISIAQ) (1996) has proposed guidelines for interpreting environmental samples for fungi. These guidelines state that in naturally ventilated, non-problem buildings, the relative abundance of different fungi in indoor air tends to follow the pattern found in outdoor air, although the numbers are usually smaller. When air-conditioning or mechanical ventilation with filtration is used, indoor fungal concentrations in non-problem buildings may be even lower than in naturally ventilated buildings. When windows are closed or when snow cover reduces outdoor sources of fungi, indoor sources of *Penicillium* spp. and other soil fungi may be more obvious. While a diversity of fungi is usually found in non-problem buildings, one or two fungal species may dominate the indoor air in buildings with persistent moisture problems. The presence or dominance of toxigenic or allergenic species indicates a problem that may cause deterioration of the quality of the indoor air.

19.5.2 Earlier ACGIH Recommendations

The last ACGIH (1989) guidelines stated that the taxa of fungi isolated from indoor and outdoor air should be similar and that the concentration of airborne fungi should be lower indoors than outdoors, the degree of difference varying with the type of building ventilation. This statement is still correct, with the understanding that in regions with winter snow cover, outdoor fungal air concentrations may be lower than indoor concentrations even in non-problem buildings It is assumed that the samples used to make such comparisons were collected appropriately and that the numbers of samples for the indoor and outdoor locations are sufficient in number to allow meaningful comparison.

Based on the authors' experience when the 1989 guidelines were written, they stated that outdoor fungal air concentrations exceeding 1000 CFU/m^3 were routine and that concentrations near 10,000 CFU/m^3 were not uncommon in summer months. Except in specialized environments where immunosuppressed persons are routinely present, levels of any saprophytic fungus below 100 CFU/m^3 were reported as not of concern. Concentration data collected since 1989 continue to support these statements about concentration ranges for total culturable or countable fungi. The earlier authors also wrote that in buildings with mechanical ventilation and minimal air filtration, indoor fungal concentrations typically are less than half of outdoor levels. Currently, it is understood that comparing the ratio of indoor and outdoor fungal concentrations requires consideration of many factors. The authors never intended the above statements to be interpreted that indoor fungal air concentrations of 50 CFU/m^3, 500 CFU/m^3, or 5000 CFU/m^3 were to be used as criteria for judging indoor air quality in various settings or different seasons. The following sections summarize ACGIH's current recommendations for evaluating fungi in indoor environments [see also Chapters 1 and 14]

19.5.3 Current ACGIH Recommendations

Rather than focusing on specific kinds of fungi or on quantitative measures of fungal prevalence, the ACGIH approach has been to emphasize that active fungal growth in indoor environments is inappropriate and may lead to

exposure and adverse health effects. Evidence that active growth is occurring is most often sensory (visual identification or odor perception) confirmed by judicious source sampling. If air sampling is to be used, ACGIH guidance has emphasized the importance of well-designed sampling protocols and reliance on carefully collected baseline data for comparison. Following is a summary of guidelines for assessing fungal problems in non-industrial indoor environments:

1. The presence of visible fungal growth confirmed by source sampling in occupied indoor environments is strong evidence that exposure may occur. The conditions leading to such growth should be corrected and the growth removed, using appropriate precautions.
2. The presence of moldy odors in occupied indoor environments is strong evidence that fungal growth is occurring. Such growth should be located and confirmed by source sampling. The conditions leading to the growth should be corrected and the growth removed, using appropriate precautions.
3. The persistent presence of water in indoor environments (except in places designed for the carriage or storage of water) is likely to lead to fungal growth. The conditions allowing such water to accumulate should be corrected.
4. The presence of accumulations of organic debris, especially bird or animal droppings, is presumptive evidence of the presence of fungal contamination. The conditions allowing the accumulation of such debris should be corrected and the debris removed, using appropriate precautions.
5. Interpretation of source or air sampling data in the absence of any of the above conditions requires a sufficient number of samples (including controls) to ensure that results are not due to random chance. If these data requirements are met, an investigator may consider sampling results in light of the following discussions.

19.5.3.1 Indoor/Outdoor Relationships Indoor/outdoor relationships are assessed both by comparing concentrations and species composition of comparably collected samples. In non-problem environments, the concentration of fungi in indoor air typically is similar to or lower than the concentration seen outdoors, except when outdoor air concentrations are near zero (e.g., during periods of snow cover). If fungal concentrations indoors are consistently higher than those outdoors, then indoor sources are indicated. However, indoor fungal growth may also be present in situations where indoor concentrations of airborne fungi are equal to or lower than those outdoors, and interpretation of data depends on a knowledge of the kinds of fungi present in the two environments. Note that exposure to fungi actively growing indoors may present unusual health risks even when total fungal concentrations are higher outdoors. If the variability of the data is high (which is common), it may be difficult to establish that two locations differ with respect to concentrations of airborne fungi [see 14.2 3 2].

The species of fungi found in indoor and outdoor air typically are similar if outdoor air is the primary source for the fungi in indoor air (Burge et al., 1977, Strachan et al., 1990, Targonski et al., 1995, AAAAI, 1996, Delfino et al., 1996, Neas et al., 1996). Comparisons of the species compositions of indoor and outdoor populations requires accurate identification of fungal species, not simply identification to the genus level. For example, a report stating that *Cladosporium* spp. predominated in both indoor and outdoor air samples may lead investigators to conclude that the indoor environment did not present a particular problem. In fact, *C. herbarum* may have been dominant outdoors and *C. sphaerospermum* dominant indoors; the former arising from outdoor sources and the latter released from areas of indoor growth. However, without identification of the fungal isolates to the species level, this possible cause of occupant complaints would be missed.

19.5.3.2 Indicator Species Fungi whose presence indicate excessive moisture or a health hazard have been termed indicator organisms. Interpreting the presence or absence of an indicator species (e g , a recognized toxigenic fungus that is uncommon in outdoor air) requires the ability to identify fungi to the species level and a knowledge of the prevalence of various fungal species in indoor and outdoor environments. The mere presence of a few CFUs or spores of an indicator species should be interpreted with caution. Identification of the presence of a particular fungus in an indoor environment does not allow investigators to conclude that building occupants are exposed to antigenic or toxic agents. Investigators should also recognize that fungi named as indicator species are not the only fungi of significance. Many fungi other than those specifically listed by various groups may cause problems for building occupants exposed through inhalation of fungal aerosols or via other contact.

19.5.3.3 Potentially Pathogenic Fungi Some fungal pathogens should be assumed to be present when materials known to support their growth are found (e.g., *H. capsulatum* and *C. neoformans* in bird and bat droppings) [see 19.2.1]. Removal of such materials should be conducted as if they contained pathogenic fungi. Disturbance of soil or other material that may contain fungal pathogens (e.g , compost containing *Aspergillus* spp or soil containing *H. capsulatum, B. dermatitidis,* or *C immitis*) should be conducted with consideration that occupants of neighboring buildings may be exposed if airborne fungal spores enter the buildings

The Facts About Mold

Operation Outreach

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EXHIBIT 31-B

This brochure is a joint effort by the following AIHA technical committees:

Biosafety and Environmental Microbiology

Environmental Microbiology Laboratory Accreditation (EMLAC)

Indoor Environmental Quality (IEQ)

AIHA

Your Essential Connection

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Introduction

Mold is in the news. People are talking about its potential health and economic impact. But what are the real risks and issues?

The available science is incomplete and sometimes controversial. Although there are several guidance documents available, there is no accepted national standard. Validated methods to measure contamination are still in their infancy, and even when measurement techniques are available, there are no clear benchmarks or standard values to compare the results against. Similar scientific uncertainties exist in the medical diagnosis of some mold-related health effects.

The scientific complexities alone would be a huge challenge, but the truth is that other difficulties dwarf them. The intense public and media attention on this topic often creates emotionally charged circumstances that make scientific judgment and reasoned dialogue difficult. In some instances, building owners tend to ignore or dismiss potentially serious problems. In other instances, building occupants or public officials can react with excessive alarm to perceived potential threats, complicating the scientific component of the evaluation and making risk communication very difficult.

While experts and practitioners disagree on which trend is of more concern, it is clear that both are real and sizable. The biggest obstacle, however, is the amount of money that can be involved in these disputes. As a result, the issue is increasingly clouded by the acrimony and distorted partisanship of mushrooming liability battles in the legal arena

This brochure represents a consensus statement by a group of experts about important aspects of the "state of the science." The guidance offered is practical information and does not claim to be a definitive or comprehensive position statement. Because it is not comprehensive, it

The Facts About Mold: For the Professional

How should a building be evaluated for mold growth? Check building materials and spaces for visible mold and signs of moisture damage indicating a history of water leaks, high humidity levels, and/or condensation Any occupant complaints or reported health problems should be noted as well as any musty or moldy odors.

Components of the building's ventilation system should also be inspected. A moisture meter is often helpful in identifying wet or damp building materials. If mold growth or moisture problems are found, the air pressure differentials between the area of growth and surrounding areas should be determined. Potential air pathways from the source should also be characterized to determine its impact on the building and its occupants.

When is sampling necessary in a building evaluation? Sampling may not be necessary. If visible mold is present, then it should be remediated, regardless of what species are present and whether samples are taken. In specific instances, such as cases where health concerns are an issue, litigation is involved, or the source(s) of contamination is unclear, sampling may be considered as part of a building evaluation. Sampling is needed in situations where visible mold is present and there is a need to have the mold identified.

If mold is suspected, but not visibly detectable after an inspection, then sampling may reveal evidence of mold amplification or reservoirs indoors. If mold is being removed and there is a question about how far the colonization extends, then surface or bulk sampling in combination with moisture readings may be useful. Sampling for airborne mold spores can indicate whether the mix of indoor molds is "typical" of the outdoor mix or, conversely, "atypical" or unusual at that time.

Professionals experienced with mold issues and familiar with current guidelines must conduct any sampling If samples are taken, regardless of the purpose, the results should help answer a clear question. Sampling without a specific purpose greatly increases the chances of generating useless data. Note that laboratories vary in experience and proficiency; using an AIHA EMLAP-accredited lab is recommended. A listing of accredited labs can be found at www.aiha.org/LaboratoryServices/html/lists.htm.

Why is there controversy about the health effects of exposure to mold growth? Not all health effects of molds are controversial Fungal infections are well known. Fungal allergies are also well known and accepted among medical experts, although the allergens themselves are poorly characterized Infections and allergies have objective and well-established clinical effects. These effects can be measured and reproducibly demonstrated, and the mechanisms are fully understood The health effects caused by consuming moldy food or feed that contains mycotoxins are also well known. Regardless of these controversies, mold growth in the built environment is unacceptable from the perspectives of potential adverse health effects and building performance

Other health effects have been proposed for mold metabolites that are irritants or mycotoxins, and plausible mechanisms exist for health effects due to these mold metabolites. However, the clinical relevance of these mycotoxins and irritants under realistic airborne exposure levels is not fully established Further, supporting evidence for other health effects is based on case studies rather than controlled studies, nonreproduced studies, or subjective symptoms.

Case studies do indicate the possibility or plausibility of an effect. Unfortunately, such studies cannot address whether an effect is common or widespread among building occupants. Results from nonreproduced studies may be false or are

Guidance for Clinicians on the Recognition and Management of Health Effects Related to Mold Exposure and Moisture Indoors

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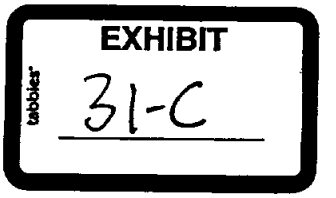


3. About Fungus and Mold

An appreciation of fungi and their ecological role will help the healthcare provider guide patients who express concern over indoor mold.[1] This section briefly identifies factors about fungi that providers should find helpful in understanding the role mold exposure may or may not have in patients' symptoms and in interpreting environmental reports.[2]

Mushrooms produced on hardwood floor where there has been long-term water incursion (Unknown or anonymous author Image courtesy of Dr Chin S Yang of P&K Microbiology Services)

Many atopic patients experience allergic symptoms related to molds commonly encountered outdoors. The presence of mold spores in the indoor environment is not in itself a problem when the source is the normal interchange of outside air and the amount and types of spores inside are the same or less than outside. However, mold actively growing on an indoor substrate may affect the quality of the environment by degrading the surrounding materials (weakening the structure) and, more important, by potentially adding unhealthy chemicals and bioaerosols to the indoor air. Higher levels of mold spores inside than outside or the presence of different species inside than outside reflect this "amplification" of mold. The next section discusses health effects that may be associated with fungi in the indoor environment.

[1] Throughout this guidance, the term "mold" is spelled according to American usage. The alternative spelling "mould" is also commonly used in literature

[2] Two helpful references used throughout this summary are chapter 19 of the American Conference of Governmental Industrial Hygienists reference *Bioaerosols. Assessment and Control* (Burge and Otten 1999) and the subchapter on biological contamination in the *Encyclopedia of Occupational Health and Safety* (Flannigan 1998).

Fungi Classification

Mycologists classify fungi by their presumed evolutionary biological relationships. The three most common groups of fungi are Zygomycetes, Ascomycetes, and Basidiomycetes. Although all can contaminate buildings, the most common fungi that colonize building materials belong to the Ascomycetes group (Burge 1997). In chapter 19 of *Bioaerosol. Assessment and Control* (Macher 1999), Burge and Otten discuss fungi as a "kingdom of eukaryotic organisms, without chlorophyll, that have cells bound by rigid walls usually formed of chitin and glucans." They further discuss that the term "mold" is an artificial grouping similar to the term "weed" used by gardeners. It has no taxonomic significance. Mold generally refers to a visible colony of fungi growing in an indoor environment. "Mildew" is a layperson's term referring to mold growing in and on substances such as fabrics and wood. This section presents a brief discussion of the morphology and ecology of fungus in the indoor environment.

Mold actively growing on an indoor substrate—resulting in indoor amplification—may affect the quality of the environment by degrading the surrounding materials, weakening the structure, and, more important, by potentially adding unhealthy fungal products and bioaerosols to the indoor air.

Ecology and Structure

Fungi are ubiquitous in the natural environment. They share characteristics of both plants and animals and are classified in a unique kingdom. Fungi can be saprophytic, parasitic, or symbiotic. Most fungi are saprophytes, and saprophytic fungi thrive by first exuding enzymes and acids that act on surrounding dead and decaying materials and then by absorbing nutrition from the breakdown, fulfilling a critical ecological role by degrading waste material.

Fungi exist in many forms: single-celled yeasts, microscopic filaments (termed hyphae), large visible mats of mycelium (an aggregate of hyphae), and visible spore-producing fruiting bodies known as basidiomycetes, which include common mushrooms. Different fungi are associated with different health effects, and specific components of fungi (such as glucans in the cell walls) or forms of the fungi (spores) are thought to be agents associated with illness.

Other Microbial Agents Indoors

It is important to note that bacteria also grow on building materials and are likely contributors with fungi of bioaerosols to the indoor environment. In a water-damaged environment, environmental bacteria such as gram-negatives and actinomycetes may amplify along with molds. The growth of environmental bacteria may also produce a variety of byproducts, such as endotoxins and bacterial volatile organic compounds (VOCs). Some bacterial species, e.g., *Pseudomonas*

aeruginosa, may cause opportunistic infections. The response to water damage by gram-negative bacteria is very rapid. In contrast, amplification of actinomycetes is often due to long-term or chronic water damage. Peltola reports that gram positive bacteria species were isolated with a toxigenic fungal species from a home where the occupant experienced substantial symptoms and the bacterial species have been shown to produce toxic metabolites (Peltola et al. 2001).

Photomicrograph of *Beauvaria bassiana*, which is relatively common indoors A natural insect parasite, *Beauvaria bassiana* has been studied as a biocontrol agent of insects It can become a significant issue indoors because of moisture problems leading to insect amplification and, hence, growth of the fungus on insects, both alive and dead (image courtesy of Dr De-wei Li of P&K Microbiology Services)

Nutrition and Growth

The type and characteristic/life stage of fungi in the environment is influenced by moisture, nutrition, light, oxygen, and temperature. In some species, light facilitates sporulation more than mycelial growth. Fungi will grow anywhere indoors and outdoors over a broad temperature range where there is sufficient moisture and a nutrient source. Most fungi prefer a temperature of 15°C-30°C (59°F-86°F), but there are varieties that will grow below or above these temperatures. For example, thermophiles have optimal growth from 35°C -50°C (95°F -122°F).

Fungi can use dirt, dust, wood, paper, paint, insulation, or other common materials for nutrition. This means mold can be established in upholstery, carpet, wall board, ceiling tiles, and even in dirt on glass. Because they are involved in the decaying process, their source of nutrient is almost any organic material, and specific species may have preferences. *Stachybotrys* prefers cellulose and grows exceptionally well on wallpaper or the paper and gypsum of wallboard. Because of these growth preferences, cultures from interior room surfaces or air do not necessarily represent the true distribution of mold in the indoor environment. When conditions are appropriate, fungi may produce secondary metabolites that may be toxic to humans and animals or other organisms.

In most indoor environments, the availability of moisture becomes the limiting factor to amplification or growth of mold. Moisture must be continually present for a colony to grow. Extensive growth has most often been associated with the presence of water in materials or condensation from high humidity, but the environment does not have to be "wet" to support mold associated with health problems. Dampness, which is noted only by minor moisture/condensate, is adequate for some mold, including species of *Aspergillus* and *Penicillium*, molds that are thought to be a problem to the health of some building occupants. Other, more hydrophilic, molds (*Stachybotrys*, *Fusarium*, and *Acremonium*) grow in higher moisture content. Moisture is referred to by mycologists in terms of water activity, i.e. the measure of water within a substrate that an

organism can use to support its growth. Optimal water activity varies according to mold species. Wall relative humidity (because it reflects water activity in the substrate) has been shown to be a better indicator of *Stachybotrus chartarum* that relative humidity (Boutin-Forzano et al. 2004).

Reproduction and Dispersal

Fungi reproduce by sexual (via meiosis) or asexual (via mitosis) means in the form of spores Fungi normally reproduce by mitosis and cell division, growing colonies. Most fungi survive undesirable conditions and disperse into the environment in spore forms. Individual spores are dispersed and then produce complete fungal organisms in response to appropriate growth conditions. Some spores are slimy and (more) easily stick to substrates, while others are powdery (dryer) and more easily aerosolized. Most spores are respirable (2-10 mm), but some spores can well exceed respirable size (100 mm). In the outside environment, mold spores are dispersed naturally in a diurnal and seasonal pattern. Without an indoor source, indoor air is often reflective of outdoor air (Burge et al. 2000). This diurnal pattern adds to the variability and difficulty in interpreting indoor air mold sampling results. When sources of mold are from the indoor environment, it is unclear how spores are dispersed. Although some spores may be released by colonies and carried by normal air currents similar to what happens in the outdoor environment, human activities inside may disperse mold spores. Reservoirs of mold spores in carpet, walls, ceilings, or furniture may very well be dispersed by any activity such as vacuuming, walking, sitting down on upholstered furniture (Chao et al. 2003), or other disturbances to the building materials.

Fungal Products

Mold products include compounds that are common to all molds, such as glucans, a major structural component, and ergosterol. These can be measured to estimate total mold burden in an environment. Molds secrete enzymes that degrade nutrient-containing substrates on which molds grow. Products of this metabolic activity may be absorbed by the mold organisms or remain in the environment. Byproducts of this metabolism are carbon dioxide, water and ethanol or lactic acid, and sometimesVOCs. The VOCs may include alcohols, esters, aldehydes, hydrocarbons, and aromatic compounds. Some fungi produce secondary metabolites. These VOCs and secondary metabolites may be responsible for the characteristic "musty" odors in buildings where molds grow.

Fungal metabolic byproducts may have toxic, allergenic, or immunologic effects. Although their role in fungal ecology is unclear, some of these substances have had specific effects on humans (Etzel 2003a). For example, fungal metabolites include important antibiotics (e.g., penicillin), potent toxins (e.g., aflatoxin) and psychoactive compounds (e.g., psilocybin) (Burge 1992). For specific fungal species, toxic metabolites may provide the organism with a competitive advantage over other species. There are hundreds of known mycotoxins, in a large variety of structural types, with different biological properties (Norred and Riley 2001). Some of these metabolites are produced by a number of unrelated species, and others are very specific. If an individual becomes allergic to a structural component or metabolite that is found across species, he or she will react

allergically to a number of different molds. Fungi produce non-volatile mycotoxins that can injure or cause the death of eucaryotic cells. Most mycotoxins are heterocyclic organic molecules, generally having molecular weights of 300-750 daltons. Animal studies have confirmed teratogenic, carcinogenic, immune-suppressive, and other associations with a variety of mycotoxins (Robbins et al. 2000). Although they are not usually volatile by themselves, mycotoxins may readily enter the air in spores and fungal fragments when the substrate is disturbed. For example, children may become exposed when playing on mold-contaminated carpet.

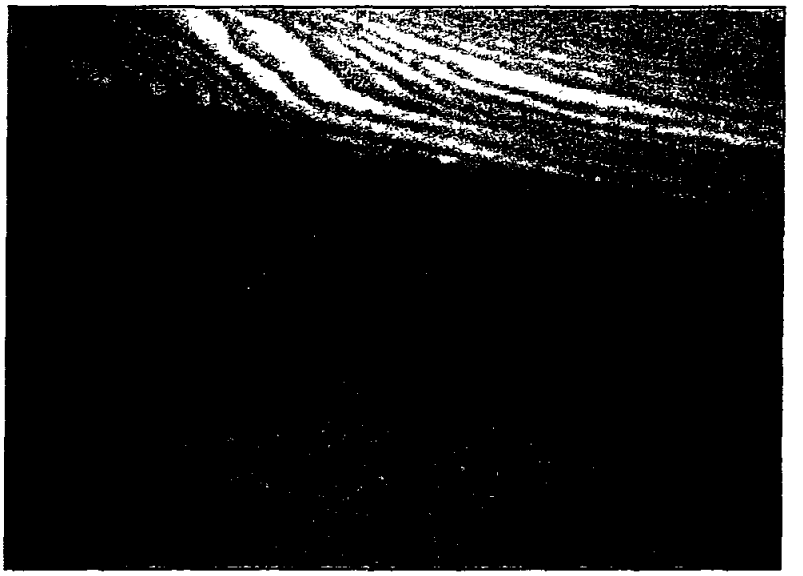
Specific Molds

Appendix A presents brief descriptions of a selected list of fungal species commonly found in the indoor environment and whose exposures may be of concern to your patients' health. They include *Aspergillus spp., Alternaria spp., Acremonium spp., Cladosporium spp., Dreschslera spp., Epicoccum spp., Penicillium spp., Stachybotrys spp.,* and *Trichoderma spp.* (Assouline-Dayan et al. 2002). Because patients may have concerns over mycotoxins in general, some species that are not commonly found in the indoor air environment, but have been shown to produce toxins, are also listed in the appendix. However, this list is not designed to cover all fungi. If interested in more information on fungi, clinicians should consult a competent mycologist or these suggested references from the mycological literature:

- *Introduction to Food- and Airborne Fungi Sixth Edition*, 2000; Samson, Hoekstra, Firsvad, and Filtenborg; The Netherlands.
- Microorganisms in Home and Indoor Work Environments: Diversity, Health Impacts, Investigation and Control, 2001; Ed: Flannigan, Samson and Miller; Taylor & Francis; London and New York.
- *The Fifth Kingdom* on CD-ROM. Version 2.5, 2001; Kendrick; Mycologue Publ, Sidney.
- *Fungal contamination as a major contributor of sick building syndrome* in *Sick Building Syndrome*; 2004; Li, Yang; Academic Press, San Diego.

6. Environmental Assessment

When exposure to mold plays a role in the patient's health, consideration of the environment becomes a key element in understanding and treating the patient's illness.



Fruiting bodies of a *Peziza sp* in a room with chronic water damage. *Peziza sp.* is an ascomycete (producing sexual ascospores in a structure called the ascus) and is also known as one of the cup fungi It is occasionally found on chronically wet or water-damaged wood products (such as plywood subfloor) *Peziza* can be identified only when the sexual fruiting body is present In culture, its vegetative state is named *Chromelosponum sp* (Unknown or anonymous author, image courtesy of Dr Chin S Yang of P&K Microbiology Services)

This chapter gives practitioners an abridged version of the principles that underlie a professional environmental assessment so they can (1) better evaluate patient information about their environments and (2) use environmental assessment as a tool to prevent mold-related illnesses and to treat individual patients presenting with symptoms and illnesses exacerbated by mold in their environment.

Resources to initiate a professional assessment are most often absent or at best limited. Consequently, when exposure to mold indoors is potentially associated with symptoms, the healthcare provider may choose to give the patient a home checklist (Table C) and a list of references (Table E) as guidance on how to minimize mold growth in his or her environment, without initiating elaborate environmental assessments to confirm the presence of mold.

The reader is referred to the following references for detailed guidance on how buildings are evaluated for bioaerosols and mold:

- *Bioaerosols: Assessment and Control*, 1999; Ed: Janet Macher, American Conference of Governmental Industrial Hygienists.

- *Mold Remediation in Schools and Commercial Buildings*, March 2001; U.S. Environmental Protection Agency; EPA 402-K-01-001.

- *Fungal Contamination in Public Buildings: A Guide to Recognition and Management,* June 1995; Federal-Provincial Committee on Environmental and Occupational Health; Health Canada.

- Microorganisms in Home and Indoor Work Environments: Diversity, Health Impacts, Investigation and Control, 2001; Ed: Flannigan, Samson and Miller; Taylor & Francis; London and New York.

When is it important to intervene in the home or work environment? The algorithm presented in chapter 5 provides guidance for the physician. An assessment of mold in the environment may become especially important for patients with specific symptoms and syndromes (see Table A in chapter 5) or for patients with other common symptoms and syndromes (see Table B and Grid D in chapter 5) that are worse in a particular environment. The reader should note that the authors do not advocate air sampling to initially address concerns over mold in the indoor environment. This is in part because air test results are often not representative of the biological exposures a patient may face and, therefore, can be misleading and not helpful. Because the health provider may be given reports and information that includes air-sampling results, this chapter provides guidance on planning an indoor air assessment for mold and on interpreting air-sampling results.

Consultant Selection and Staff Training

Patients may bring healthcare providers reports with contributions from different types of professionals, including specialists in ventilation, industrial hygiene, environmental science, architecture and building physics, occupational and environmental medicine, mycology, and public health. To evaluate and then use the information in these assessments, it is critical to know the context of the assessment and the background and credentials of the individuals who performed them. The US EPA provides guidance on hiring assistance for indoor air quality assessment and remediation in various programs: the I-Beam Visual Reference Index (www.epa gov/iaq/largebldgs/qref_frame.htm), Indoor Air Quality Tools for Schools (EPA Tools for Schools Kit,www.epa.gov/iaq/schools/tfs/guidea.html) and Indoor Air Quality in Large Buildings (Building Air Quality, www.epa.gov/iaq/largebldgs/graphics/sec_8.pdf).

For the healthcare provider who may look to suggest an outside environmental assessment, the following paragraphs briefly discuss three categories of professionals who will most likely bring a learned approach to the challenge of assessing the environment for exposures to bioaerosols: industrial hygienists, indoor environmental quality consultants, and environmental health professionals. Although not as common, other professionals may provide assessments or specialized expertise to address indoor environments. **Experience conducting environmental assessments with a focus on bioaerosols** is a key qualification for any of these professionals.

Frequently, it is also helpful to consult with mycologists and building scientists. Mycologists knowledgeable about indoor-mold-contamination issues bring a critical perspective to designing sampling programs and interpreting results. Building scientists (usually architects or engineers who have specialized expertise) bring helpful skills and an understanding of the movement of moisture and air in the building, which are often instrumental in finding and remediating moisture intrusion.

Industrial Hygienists

In the broadest sense, an industrial hygienist focuses on exposures that affect the health and well-being of workers. These individuals are well versed in measuring and assessing occupational hazards. The American Board of Industrial Hygiene (ABIH) certification program requires a bachelor's degree in an associated field (usually engineering or one of the natural sciences), passage of an examination covering a broad range of relevant subjects, and a minimum of 5 years' experience in some association with a practicing Certified Industrial Hygienist (CIH). The ABIH had offered certification in indoor air quality; however that certification is no longer available. Certified Industrial Hygienists have the training to develop the broad perspective required to address mold in the environment. However, because (1) exposure to bioaerosols is not readily identified by standard air-monitoring methods, (2) home and office environments are different than industrial sites, and (3) the biology of mold is complex, an assessment is best completed by an industrial hygienist **experienced with mold assessment**.

A qualitative assessment that identifies factors that support the growth of indoor fungi and makes recommendations for correcting these factors provides helpful guidance for the healthcare provider and the patient. Measurements of fungal colonies and spore counts are not as helpful.

Indoor Environmental Quality (IEQ) Consultant.

Individuals who practice as indoor environmental/air quality consultants come from many different backgrounds (engineering, basic sciences, planning, and design) and different professions (ventilation, building engineering, industrial hygiene, environmental science, construction, and architecture). Some IEQ consultants bring an appreciation of agents in the environment and exposure because they have worked on environmental problems with a concern for health impacts. However, other environmental professionals, though competent in their individual expertise, lack either the broad health perspective or specific knowledge regarding bioaerosols needed for an adequate assessment when mold may be an issue. For example an IEQ consultant may have specialized experience with ventilation systems, but lack an understanding of sources and distribution of bioaerosols in the environment. As with Certified Industrial Hygienists, IEQ consultants who are **experienced in determining exposures from mold in the environment** provide the better assessments.

Environmental Health Professional

Because an understanding of the building occupants' illnesses and symptoms has become critical to appropriately focus the investigation in many situations, environmental health professionals have assumed an active role in environmental assessment. Occupational and environmental medicine physicians and nurses, as well as public health professionals (Masters in Public Health and graduate-level epidemiologists), bring relevant background to environmental assessment. The patterns and locations where occupants experience symptoms help direct where to look for mold sources. Moreover, the local health director or state official is not infrequently the person who directs or orders an environmental assessment in a public building, such as a school, when poor indoor environmental quality is suspected because of a high level of health complaints.

Patient or Family Member as Investigator of Environment

The patient or a family member may assess the environment for mold. One caution: if you suspect mold is present and may be playing a role in illness and you direct your patient to investigate his or her environment beyond the home checklist, it would be prudent to suggest that the patient use care when exploring his or her environment. If the individual develops symptoms while investigating, he or she should be cautioned to ask someone else to explore for and clean up mold contamination if needed. Guidance on personal protection and how to remediate mold contamination is addressed in the next chapter of this book.

Qualitative Approach to Environmental Site Assessment

A qualitative assessment that identifies factors that support the growth of indoor fungi and makes recommendations for correcting these factors provides helpful guidance to the healthcare provider. We use the term "assessor" to identify the individual conducting the evaluation. The assessor can be the patient, a family member, or a professional

The environmental assessor seeks to identify sources of mold growth (reservoirs) and to define the pathways in the environment that may bring mold and any associated toxins into contact with the building occupants (Burge and Otten 1999). The objective is to find areas where mold is amplified (growing) and then disseminated into the breathing space. Normally, people should not see or smell mold or mildew in their indoor spaces. A moldy odor or visible evidence of mold colonies or mildew on materials indicates the presence of mold. However, mold may be present even if not smelled or seen.

Interview and Walk-through Assessment

The assessor gathers qualitative data by interviewing the occupants and taking a walk-through site tour. If the assessor is the patient, noting where in the home environment and under what conditions (such as heat on or off) he or she experiences symptoms will indicate where to look. The walk-through will explore the immediate outside environment and the physical structure of the home or building; note water or moisture incursion from past and present leaks, spills, and condensation; review ventilation and

note apparent mold, mildew, and areas with moldy, musty odors. Likely places where moisture may accumulate, such as crawlspaces, should be noted.

Focused Qualitative Assessment

The assessor will minimally address the following (adapted from Macher 1999, Health Canada 1995):

- A review (visual assessment) of the immediate outside environment and building exterior for:
  - Sources of outside molds (for example, leaf piles).
  - Damage to the building (roof, wall, windows and foundation), especially damage that would allow water intrusion.
  - Accumulations of organic material in or near air intakes (e.g., bird or bat droppings because they support the growth of pathogenic fungi and plant material that generally supports fungal growth).
  - Grading (poor drainage and below-grade air intakes or basement windows).
  - Evidence of standing water where it may be affecting the indoor environment.

- Heating, ventilation, and air-conditioning (HVAC) system assessment of:
  - Filters (dampness and microbial growth, dirt).
  - Heat exchangers (e.g., cooling coil section including drain pan), ductwork, and air diffusers (for dampness, microbial growth, dirt, and rust).

- Occupied space survey of:
  - Water damage (leaks, high humidity, musty or moldy odors).
  - Chronic condensation (typically cool surfaces such as outside walls and windows).
  - Air conditioners (standing water, microbial growth, dirt).
  - Carpet (for evidence of water damage).
  - Other fabric materials such as upholstery, furniture, and drapes (for dampness, microbial growth, and dirt).
  - Portable humidifiers (for standing water, microbial growth, and dirt).
  - Plants (for mold growth on dirt and on plants and for water damage on flooring beneath pots).

Ventilation System Review

Because the way air moves in the building and the condition of the HVAC system, if present, are critical aspects of bioaerosol exposure, a systematic review of the mechanical ventilation system should be part of the initial walk-through assessment. Indoor environments are ventilated with different systems. For example, the simplest system may be operable windows that allow outside air into homes and buildings. More complex ventilation will use central intakes to bring in air, filter and condition it, and then disburse the conditioned air into the space. This section describes key elements of reviewing ventilation systems and is followed by a brief discussion of home ventilation.

Ventilation systems in buildings often operate differently from the design specifications when they were first engineered. Because the amount and quality of the air flowing through the system can be of critical importance to the indoor air quality, a qualitative assessment of the ventilation system is a key aspect of assessing the patient's environment.

Ventilation with outside air can dilute the concentration of indoor contaminants. Mechanical ventilation systems should be properly maintained to optimize the volume of dilution air and to minimize the accumulation of contaminants, specifically microbial growth, within the ventilation systems themselves. Ventilation systems can supply buildings with tempered and dehumidified outside air. It is important to note, however, that ventilation effectively dehumidifies buildings only when the outdoor air dew point is less than 55°F. Above dew points of 70°F or so, ventilation is likely to become the dominant source of indoor water vapor.

Although mechanical ventilation systems have varying design characteristics, the following approach can be followed to qualitatively evaluate the system's cleanliness from a microbial growth perspective. When evaluating a ventilation system, it is helpful to have the assistance of the building's maintenance or mechanical engineering personnel. These individuals can provide access to the unit, are familiar with the unit's maintenance history, and can describe the system's design parameters.

Mechanical ventilation systems should supply buildings with outside air. As part of a ventilation system evaluation, the assessor should identify the location of the outside air intake. These intakes should be at least 20 feet from potential microbial reservoirs such as cooling towers, standing water, and gutters filled with leaves, pigeon droppings, or other organic material. Because all outside air contains bioaerosols, ventilation systems should have efficient filters that can remove some of this material from the incoming air stream. These filters should be replaced regularly (ideally quarterly) as part of a preventive maintenance program.

Once the outside and/or recirculated air passes through a bank of filters, it may be tempered by passing over either heating or cooling coils. Because cooling coils remove moisture from the air stream, a drain pan should be located below the coils to collect condensate. This pan should be sloped to prevent the build-up of standing water and microbial growth in the pan. If the ventilation system is designed to humidify the air — not recommended unless special circumstances call for humidification — care should be taken to prevent the humidification system itself from becoming a microbial reservoir and amplifier.

The condition of the filters and the drain pan can be evaluated visually by opening the air-handling unit when the system is not in operation.

After the air has been tempered, it may pass through a series of ducts until it is distributed to the occupied spaces. A visual assessment of the ductwork may be possible through access panels.

Ducts without internal lining are desirable. Ducts with internal lining or duct board can become microbial reservoirs and amplifiers if they become humid and dirty. A combination of internal fiberglass insulation and condensate water blowing off the cooling coil causes the most extensive mold growth in ducts.

Environmental assessment in homes focuses on good maintenance practices to ensure dirt and moisture do not accumulate and to provide adequate ventilation.

Ventilation in Homes

Outdoor air enters and leaves a house by infiltration, natural ventilation, and mechanical ventilation. Most home heating and cooling systems, including forced air heating systems, do not mechanically bring fresh air into the house. A home's ventilation rate can be increased by opening windows and doors, operating window or attic fans when the weather permits, and running a window air-conditioner with the vent control open. Environmental assessment in homes focuses on good maintenance practices to ensure dirt and moisture do not accumulate and to provide adequate ventilation.

When concerned about a patient's symptoms that may be related to exposure to bioaerosols in the home, the clinician should inquire about the home's air handling systems and maintenance. Before the heating season, forced air heating systems should be inspected and, if necessary, cleaned. Before the cooling season, several components of the central air conditioning system should be cleaned. Bushes and vegetation should be trimmed around the outside condenser unit and the coil and fan should be cleaned. The system's filters should be replaced or cleaned several times per season and the condensate drain should be regularly checked to ensure that it is carrying off excess moisture.

When mold is a concern, a good initial assessment notes:

- **Water damage** (from leaks, high humidity) and any **musty or moldy odors**.
- **Chronic condensation** (typically cool surfaces-outside walls, windows) and any **standing water** possibly from air conditioners, humidifiers.
- **Carpet condition** (especially any sign of water damage and age).
- **Condition of fabric and porous materials** such as upholstery, furniture, drapes, ceiling tiles, partitions, books (again, dampness and microbial growth, dirt).
- **Plants** (mold growth on dirt; consistent water spillage).

A window-installed air conditioner has the same components as a central system. Routine upkeep of these units should include keeping the filters and coils clean. In addition, the condenser coil and the intake vents should be free from obstruction and the condensate drain outlet should be kept unplugged and positioned away from the house.

Summary of Qualitative Assessment

The assessor will evaluate the information gathered from the walkthrough, interviews, and ventilation review. If the information is adequate, the assessor may identify how the patient has become exposed to

mold in the environment and may suggest changes to the environment to limit the exposure. When there is evidence of moisture incursion, a good assessor suggests that the causes for the unplanned moisture be fully investigated and fixed, mold present on nonporous materials be cleaned, and all repeatedly wetted, water damaged porous materials be discarded. Often the qualitative evaluation is sufficient to begin planning appropriate improvements to the environment that will limit the patients' exposures from microbial growth.

When there is evidence of moisture damage, the causes of moisture intrusion should be fully investigated and fixed, mold present on nonporous, easily accessible materials cleaned, and other damaged materials discarded.

Sampling and Analysis

During some walk-through assessments, the assessor may have determined that water or dampness has provided an environment conducive to mold growth, but the assessor may be unsure about the extent of the mold contamination. If building-related illness is strongly suspected, mold is thought to be a potential problem for the patient, and there is insufficient information to broadly suggest where mold is growing, the assessor may need to implement a well-planned program of sampling and microscopic analysis in order to develop information on which to base guidance on appropriate intervention in the environment.

A well-thought-out sampling plan is the first step. The plan should reflect an understanding of the purposes of the investigation, the characteristics of mold, and the potential for exposure, along with an understanding of pathways and the limitations of both sampling and laboratory techniques. With the intent to determine exposures, when, where, and how the environment is sampled are critical to producing useful information. The quality of the results also depend on the education and training of the analyst and quality of the mycology laboratory. At the end of appendix A we have included charts summarizing air-sampling methods, source-sampling methods, and analytical methods that the healthcare provider may find helpful when navigating technical reports with indoor mold sampling results.

Generally, the assessor may use two types of sampling: *source sampling* of materials where mold may be growing (such as wood, carpets, wallboard, and adhesives on wallpaper) using swabs,wipes, or adhesive tapes and *air sampling*, where a standard volume of air is passed through a filter or impacted on growth media plates or greased microscopic slides to collect mold and spores. The American Conference of Governmental Industrial Hypienists (ACGIH) guidance *Bioaerosols Assessment and Control* (Macher 1999) *and Microorganisms in Home and Indoor Environments* (Flannigan et al. 2001) discuss sampling protocols. A competent mycologist should be consulted, especially when you are uncertain as to the specific mold species or molds likely to be present in the indoor environment.

Source Sampling and Microscopic Assessment

Once moisture becomes available, mold will grow on a variety of substrates normally found in our indoor environments. Although mold growth may not be evident by visual inspection, the assessor with microbiological training will often confirm mold growth on materials with a tape sample This microscopic examination of the residue picked up by clear tape may indicate the type of mold present. For comparison, the assessor will sample areas not indicating moisture or mold.

Bulk and Settled Dust Sampling and Microbial Culturing

The assessor may collect bulk samples of suspected mold-contaminated materials or collect dust from the materials to be analyzed for mold, as well as other allergens. The results will identify levels and dominant species, which will help the assessor characterize the burden of mold from the particular source sampled.

Air Sampling

A qualitative assessment, as outlined in this chapter, is often more valuable than air sampling to determine whether there is likely exposure to problem mold. This is because colonies of mold isolated from sampled air do not identify an unhealthy environment. More important, the failure of mold colonies to develop from sampled air does not indicate a healthy environment.

There is substantial natural variability in the amount of mold in air. Understandably, the EPA and other government agencies have not set numeric standards for indoor concentrations of mold or mold spores.

Mold is measured in air samples as colony forming units per cubic meter of air (CFU/m^3) by culturing. (Techniques to assess for mycotoxins and mold components, such as ergosterol and beta-1,3-glucans, are available and useful in a research setting .) Most often the assessor will use volumetric samplers to capture a specific volume of air and allow it to pass by a plate with the appropriate nutrient media so that, when incubated properly at a laboratory, any viable and culturable spores present will grow into mold colonies that can be identified and counted. Malt extract agar is typical for a general fungal population, but when *Stachybotrus chartarum* is suspected, cornmeal agar or Czapek cellulose agar is more appropriate. Because this technique samples the air for a short time (most often 1-8 minutes) in one discrete location, plus the fact that there is considerable spatial and temporal variation of airborne fungi, the number, time, and location of samples are critical to data quality. Appropriate reference samples are also required, because the results are often meaningful only in relation to the outdoor environment.

Another partially quantitative approach is to collect spores on membrane filters or slides. Spores are counted and provide some information about the type of fungal spores present. These "spore trap" techniques can estimate the burden of mold in environments that are (heavily) contaminated. Because they require less time than standard air sampling, where incubation often requires multiple days or weeks, spore trap techniques can be helpful in screening.

Several Polymerase Chain Reaction (PCR) technologies to detect and quantify fungi and bacteria have been developed, including a technology patented by the US EPA research laboratories in Cincinnati, Ohio (US EPA 2004). The measurement tool is based on the *in vitro* exponential amplification of species-specific DNA sequences so that they can be detected using fluorescent spectrometry. The technology is called Real-Time Polymerase Chain Reaction (Real-Time PCR) or Quantitative PCR (QPCR). The QPCR technology is very sensitive and requires exceptionally good laboratory practice to minimize cross-contamination and false-positives. Several laboratories have licensed and commercialized the technology. The use and application of the technology as a tool in mold testing and assessment is in the early stage. In order to fully understand the principles and details of the technology when reviewing and interpreting results, practitioners may want to discuss the technology with an experienced professional.[1]

Limitations and Difficulties with Mold Concentration Standards

Establishing standards based on fungal concentration threshold levels may appear reasonable at first glance, but this assumption is fundamentally incorrect. Based on fungi ecology, our current knowledge of health effects associated with fungal exposure, and basic environmental assessment and industrial hygiene principles, not enough is well understood about the short- and long-term dose-response relationships, fungal concentration variability over time, and toxic effects of fungal elements to support a standard.

Quantification of bioaerosols and their active components in the indoor environment may be a necessary element of research programs. Nevertheless, the cost and complexity of meaningfully interpreting air-sampling data limit their utility in patient care.

Interpretation of Air-sampling Data

An environmental assessor will review air data carefully to determine if there is mold growth or amplification and if species that might merit added concern are present. Methods for sampling have limitations, and the ecology of fungi and mold complicates sampling. (Fungi are ubiquitous in the environment, characterized by multiple forms, may integrate into substrate materials, and follow seasonal and diurnal patterns)

The review and interpretation of air sampling results is fraught with complexity.

The healthcare provider should review the results of air sampling with an understanding of this difficulty. The ACGIH (Macher 1999) and Health Canada (Health Canada 1995) provide detailed guidance on interpreting air-sampling data. In summary, these references suggest:

[1] Although this discussion addresses environmental samples, PCR technology has been used to detect *Aspergillus fumigatus* in rabbit lung tissue and bronchial lavage fluid If this PCR assay technique proves applicable to humans, it may have utility in diagnostic evaluation for pulmonary aspergillosis (O'Sullivan et al 2003)

- Mold indoors should reflect the outside species and the movement of outside air into the indoor environment. Mold identified in air sampled indoors should be at lower concentrations and of similar types to molds identified in air sampled from the outside. If the concentration inside is higher or the species different from the outside air, mold is suspected to be growing (amplifying) inside.[2]

- A specific species (other than, perhaps, species that may reflect a particular outside type dominant in certain climates at certain times) should not dominate the mold in the indoor air. If other species occur as a significant percentage indoors, and they do not correspond to outdoor relationships, an indoor source of the species is more probable.[3]

There is an allure to establishing a fungal concentration standard for indoor air to guide decisions. However, threshold levels of fungal concentrations in the indoor air have not been established and with our current knowledge would not be helpful in understanding exposure risk to patients.

- It is important to explore for indoor moisture and areas where the mold may be growing if certain toxigenic or highly allergenic molds—species of *Stachybotrys*, *Aspergillus*, *Penicillium*, and *Fusarium*, for example—are confirmed in the indoor air and are more dominant than in the outside samples. **Remediation should not be based on air sampling alone, however, even if these certain species are present in the sampling results.**

- Air sampling is limited, and negative results do not document the absence of mold exposure. For example, mold may be growing in carpets or on walls and wallpapers, yet not be airborne at the time of the sampling. Where there are other indications, such as moisture noted where it should not be, further investigation for hidden sources is indicated

Additional Quantitative Approaches

We began this chapter emphasizing that, with concern over bioaerosol exposure, a good assessor will begin with a qualitative assessment to identify sources of moisture in the indoor space, and we conclude by noting two quantitative approaches directed at moisture that may be helpful additions. Haverinen and colleagues published a model demonstrating that moisture characterized by location and

[2] Readers who would like to review individual case studies for examples of one scientific approach to interpreting data should see chapter 4.4 in Flannigan, Samson, and Miller (Morey 2001)

[3] For an example, the reader may refer to a study in which fungal profiles inside buildings (where occupants had health complaints) tended to remain unchanged with *Penicillium sp* dominant, while outdoor concentrations changed continuously over 6 hours (McGrath et al. 1999)

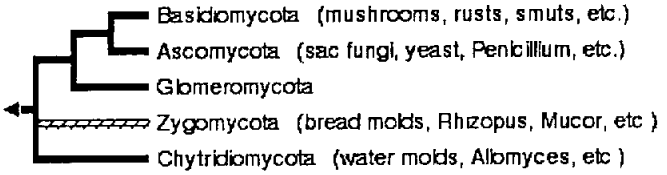
size of damage, duration of presence, and type of damage and material correlated with health symptoms (Haverinen et al. 2001). This suggests that measurements of the area of moisture damage may provide useful information in environmental assessment.

With water as the critical limiting factor for mold growth, measurements of temperature and relative humidity (RH) in the room and (when growth on building material is suspected) in the walls may be helpful to indicate water activity. (Water activity is the measure of water available within a substrate that an organism can use to support its growth.) High relative humidity in the walls was shown to correlate well with *Stachybotrys chartarum* growth (Boutin-Forzano et al. 2004). When a source of growth is indicated but not apparent, RH measurements may help direct the assessor to sampling locations and minimize the need for destructive sampling and the taking of unnecessary bulk samples.

Fungi

Eumycota mushrooms, sac fungi, yeast, molds, rusts, smuts, etc

Meredith Blackwell, Rytas Vilgalys, and John W. Taylor



Phylogeny modified from Bruns et al. 1991, 1993

Containing group: Eukaryotes

Introduction

The organisms of the fungal lineage include mushrooms, rusts, smuts, puffballs, truffles, morels, molds, and yeasts, as well as many less well-known organisms (Alexopoulos et al., 1996). About 70,000 species of fungi have been described; however, some estimates of total numbers suggest that 1.5 million species may exist (Hawksworth, 1991; Hawksworth et al., 1995).

As the sister group of animals and part of the eukaryotic crown group that radiated about a billion years ago, the fungi constitute an independent group equal in rank to that of plants and animals. They share with animals the ability to export hydrolytic enzymes that break down biopolymers, which can be absorbed for nutrition. Rather than requiring a stomach to accomplish digestion, fungi live in their own food supply and simply grow into new food as the local environment becomes nutrient depleted.

Most biologists have seen dense filamentous fungal colonies growing on rich nutrient agar plates, but in nature the filaments can be much longer and the colonies less dense. When one of the filaments contacts a food supply, the entire colony mobilizes and reallocates resources to exploit the new food. Should all food become depleted, sporulation is triggered. Although the fungal filaments and spores are microscopic, the colony can be very large with individuals of some species rivaling the mass of the largest animals or plants.

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tabbies 31-D

Figure 1: Hyphae of a wood-decaying fungus found growing on the underside of a fallen log. The metabolically active hyphae have secreted droplets on their surfaces

Prior to mating in sexual reproduction, individual fungi communicate with other individuals chemically via pheromones. In every phylum at least one pheromone has been characterized, and they range from sesquiterpines and derivatives of the carotenoid pathway in chytridiomycetes and zygomycetes to oligopeptides in ascomycetes and basidiomycetes.

Within their varied natural habitats fungi usually are the primary decomposer organisms present. Many species are free-living saprobes (users of carbon fixed by other organisms) in woody substrates, soils, leaf litter, dead animals, and animal exudates. The large cavities eaten out of living trees by wood-decaying fungi provide nest holes for a variety of animals, and extinction of the ivory billed woodpecker was due in large part to loss, through human activity, of nesting trees in bottom land hardwoods. In some low nitrogen environments several independent groups of fungi have adaptations such as nooses and sticky knobs with which to trap and degrade nematodes and other small animals. A number of references on fungal ecology are available (Carroll and Wicklow, 1992; Cooke and Whipps, 1993; Dix and Webster, 1995).

However, many other fungi are biotrophs, and in this role a number of successful groups form symbiotic associations with plants (including algae), animals (especially arthropods), and prokaryotes. Examples are lichens, mycorrhizae, and leaf and stem endophytes. Although lichens may seem infrequent in polluted cities, they can form the dominant vegetation in nordic environments, and there is a better than 80% chance that any plant you find is mycorrhizal. Leaf and stem endophytes are a more recent discovery, and some of these fungi can protect the plants they inhabit from herbivory and even influence flowering and other aspects of plant reproductive biology. Fungi are our most important plant pathogens, and include rusts, smuts, and many ascomycetes such as the agents of Dutch elm disease and chestnut blight. Among the other well known associations are fungal parasites of animals. Humans, for example, may succumb to diseases caused by Pneumocystis (a type of pneumonia that affects individuals with supressed immune systems), Coccidioides (valley fever), Ajellomyces (blastomycosis and histoplasmosis), and Cryptococcus (cryptococcosis) (Kwon-Chung and Bennett, 1992).

Figure 2. The fluffy white hyphae of the mycorrhizal fungus Rhizopogon rubescens has enveloped the smaller roots of a Virginia pine seedling. Note that some of the mycelium extends out into the surrounding environment. Copyright © J B Anderson 1996.

Figure 3 Entomophthora, "destroyer of insects", is the agent of a fungual infection that kills flies. After their death the fungal growth erupts through the fly cuticle, and dispersal by forcible spore discharge is a source of inoculum for infection of new flies. Copyright © G. L. Barron 1996

Fungal spores may be actively or passively released for dispersal by several effective methods. The air we breathe is filled with spores of species that are air dispersed. These usually are species that produce large numbers of spores, and examples include many species pathogenic on agricultural crops and trees. Other species are adapted for dispersal within or on the surfaces of animals (particularly arthropods). Some fungi are rain splash or flowing water dispersed. In a few cases the forcible release of spores is sufficient to serve as the dispersal method as well. The function of some spores is not primarily for dispersal, but to allow the organisms to survive as resistant cells during periods when the conditions of the environment are not conducive to growth.

Fungi are vital for their ecosystem functions, some of which we have reviewed in the previous paragraphs. In addition a number of fungi are used in the processing and flavoring of foods (baker's and brewer's yeasts, Penicillia in cheese-making) and in production of antibiotics and organic acids. Other fungi produce secondary metabolites such as aflatoxins that may be potent toxins and carcinogens in food of birds, fish, humans, and other mammals.

A few species are studied as model organisms that can be used to gain knowledge of basic processes such as genetics, physiology, biochemistry, and molecular biology with results that are applicable to many organisms (Taylor et al., 1993). Some of the fungi that have been intensively studied in this way include Saccharomyces cereviseae, Neurospora crassa, and Ustilago maydis.

Most phyla appear to be terrestrial in origin, although all major groups have invaded

marine and freshwater habitats. . exception to this generality is the flagellum-bearing phylum Chytridiomycota, which probably had an aquatic origin; however, some chytrid species do occur in terrestrial environments, primarily as plant pathogenic fungi.

Characteristics

Fungi are characterized by non-motile bodies (thalli) constructed of apically elongating walled filaments (hyphae), a life cycle with sexual and asexual reproduction, usually from a common thallus, haploid thalli resulting from zygotic meiosis, and heterotrophic nutrition. Spindle pole bodies, not centrioles, usually are associated with the nuclear envelope during cell division. The characteristic wall components are chitin (beta-1,4-linked homopolymers of N-acetylglucosamine in microcrystalline state) and glucans primarily alpha-glucans (alpha-1,3- and alpha-1,6- linkages) (Griffin, 1994).

Figure 4: Portion of a hypha of a zygomycete stained with a blue dye to show the many nuclei present. Many other fungi have septations that devide the hyphae into compartments that usually contain one to several nuclei per compartment. Copyright © M. Blackwell 1996

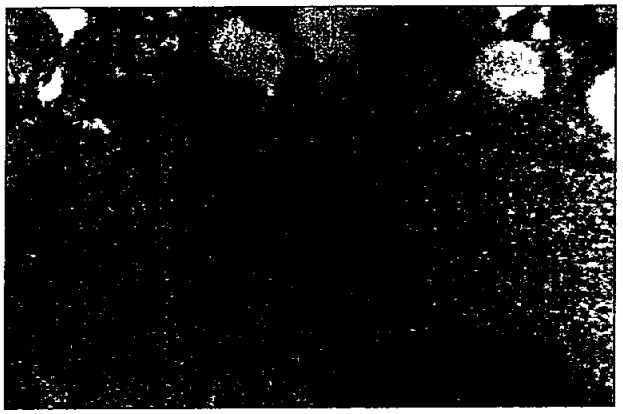

Figure 5: Transmission electron micrograph showing duplicated spindle pole body of a prophase I meiotic nucleus of a basidiomycete Exobasidium. Only chytrids among fungi have centrioles and lack spindle pole bodies. Copyright © Beth Richardson 1996.

Exceptions to this characterization of fungi are well known, and include the following: Most species of Chytridiomycota have cells with a single, smooth, posteriorly inserted flagellum at some stage in the life cycle, and centrioles are associated with nuclear division. The life cycles of most Chytridiomycota are poorly studied, but some (Blastocladiales) are known to have zygotic meiosis (therefore, alternation between haploid and diploid generations). Certain members of Zygomycota, Ascomycota, and Basidiomycota may lack hyphal growth during part or all of their life cycles, and, instead, produce budding yeast cells. Most fungal species with yeast growth forms contain only minute amounts of chitin in the walls of the yeast cells. A few species of

Ascomycota (Ophiostomataceae, ave cellulose in their walls, and certain mem. .s of Chytridiomycota (Coelomomycetales) lack walls (Alexopoulos et al., 1996).

Fossil Record

Based on the available fossil record, fungi are presumed to have been present in Late Proterozoic (900-570 mya). Terrestrial forms of purported ascomycetes are reported in associations with microarthropods in the Silurian Period (438-408 mya) (Sherwood-Pike and Gray, 1985). Fossil hyphae in association with wood decay and fossil chytrids and Glomales-Endogenales representatives associated with plants of the Rhynie Chert are reported from the Devonian Period (408-360 mya) (Hass et al., 1994; Remy et al., 1994a, 1994b; Taylor et al., 1994a, 1995b). Fungal fossil diversity increased throughout the Paleozoic Era (Taylor et al., 1994b) with all modern classes reported in the Pennsylvanian Epoch (320-286 mya).

A first attempt to match molecular data on fungal phylogeny to the geological record shows general agreement, but does point out some conflicts between the two types of data (Berbee and Taylor 1993).

Biogeography

Wherever adequate moisture, temperature, and organic substrates are available, fungi are present. Although we normally think of fungi as growing in warm, moist forests, many species occur in habitats that are cold, periodically arid, or otherwise seemingly inhospitable. It is important to recognize that optimum conditions for growth and reproduction vary widely with fungal species. Diversity of most groups of fungi tends to increase in tropical regions, but detailed studies are only in their infancy (Isaac et al., 1993).

Although many saprobic and plant pathogenic species with low substrate specificity and effective dispersal systems have broad distributions, gene flow appears to be restricted in many fungi. For these species large bodies of water such as the Atlantic and Pacific Oceans create barriers to gene exchange. Some distributions are limited by substrate availability, and dramatic examples come from parasites of Gondowanan plants; one of these is the Southern Hemisphere distribution of the ascomycete Cyttaria, corresponding with part of the distribution of its host plant Nothofagus. The fossil record shows that fungi were present in Antarctica, as is the case for other organisms with Gondwanan distributions. Arthropod associates also may show distributions throughout part or all of a host range, and some fungal species (ex. wood wasp associates) occur outside the range of the associated arthropod.

Notable Fungi

- The largest basidiocarp known is that of a Rigidioporus ulmarius in a shady, hidden-away corner of the Royal Botanic Gardens, Kew, Surrey, England. This basidiocarp is mentioned in the Guinness Book of Records (Matthews, 1994). At the beginning of each new year the Annual Mensuration Ceremony of the basidiocarp takes place. On 19 January 1996 the basidiocarp had increased to 170 cm maximum length (up from 159 in 1995) and 146 cm maximum width (up from 140 in 1995). It also grew 4 cm taller from the soil level, now measuring 54 cm. The weight of the basidiocarp has been estimated to be 284 kg (625 pounds)! Other large basidiocarps are those of a puffball almost 9 feet in circumference in Canada (over 48 pounds) and a basidiocarp of the sulfur mushroom in England (100 pounds).

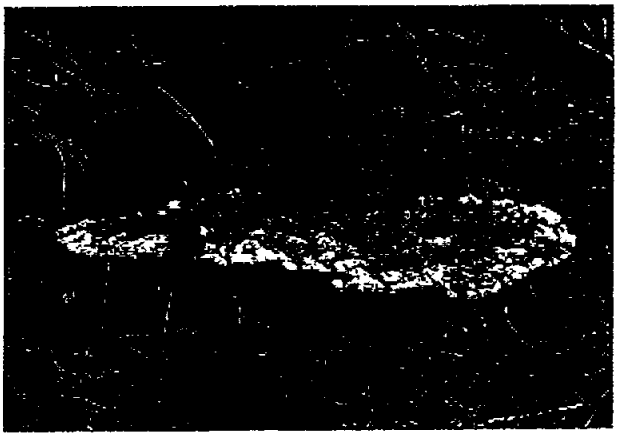

Figure 6: Largest basidiocarp world record holder Rigidioporus ulmarius at Kew. The basidiocarp is shown in its largest dimension (170 cm or over 5 1/2 feet). Copyright © D. Pegler 1996.

- Reproductive structures clearly can be very large, but what about the body of the fungus, which often is hidden from view within the substrate? One fungus body constructed of tubular filaments (hyphae) was brought to our attention when molecular techniques were used to show that it was extensive (37 acres and an estimated blue whale equivalent size of 110 tons). The Michigan fungus clone (Armillaria bulbosa) grew in tree roots and soil. This report drew attention to an even larger fungal clone of Armillaria ostoyae, reported earlier in the state of Washington, which covered over 1,500 acres. Each clone began from the germination of a single spore over a thousand years ago. Although they probably have fragmented and are no longer continuous bodies, such organisms give us cause to think about what constitutes an individual.
- Penicillium chrysogenum is known for its production of the antibiotic penicillin. Although other antibiotics are produced by a variety of organisms, penicillin was the first to be developed. In the spring of 1996 a long dried out culture of the original isolate prepared by its discoverer, Sir Alexander Fleming in the late 1920s, was auctioned by Sotheby's of London and sold to a pharmaceutical company for 23 000 pounds. This price is insignificant when one considers the worth of this fungus, not only in sales of penicillin, but in terms of illnesses cured and lives saved. In the past a simple scratch sometimes could produce a fatal infection such as the one that resulted in the death of Tad Lincoln, the son of a U. S. president. However, misuse of penicillin and other antibiotics has resulted in selection of resistant microorganisms, and the threat of untreatable bacterial infections and diseases (for example, tuberculosis and syphilis) has returned.
- Fungal spores fill the air we breathe. On many days in some localities the number of fungal spores in the air far exceeds the pollen grains. Fungal spores also cause allergies; however, unlike seasonal pollen production, some fungi can produce spores all year long. The largest number of fungal spores ever sampled was over 5.5 million per cubic foot in Wales (Matthews, 1994).
- Basidiomycetes have always attracted a lot of attention because some of them have large basidiocarps, but the realization that all fungi are important in ecosystem function has drawn more attention to microscopic forms as well. For example a report on the secret sex life of a yeast-like ascomycete human pathogen, Coccidioides immitis, made a headline of the New York Times (6 February 1996, p. B7). This fungus causes Valley Fever and is endemic in parts of the southwestern United States. Although no one has been able to observe sexual reproduction in this species, molecular studies show genetic diversity that is best explained by occurrence of sexual reproduction in the life cycle.
- Another yeast-like ascomycete reported in the Dallas Morning News (28 August 1995, p. 8D) lives in the gut of cigar beetles and is essential to the beetle's health. Without the gut fungi to detoxify the plant material of toxins, the beetles would be poisoned. Keep on the lookout for other reports of fascinating fungal feats.

Discussion of Phylogenetic Relationships

As presently delimited, the kingdom Fungi is believed to constitute a monophyletic group that shares some characters with animals such as chitinous structures, storage of glycogen, and mitochondrial UGA coding for tryptophan. Only species of the basal Chytridiomycota possess the primitive character of a single smooth, posteriorly inserted flagellum (Barr, 1992; Cavalier-Smith, 1987, 1995).

The branch uniting the fungi and animals is well-supported from nuclear small subunit ribosomal RNA gene (SSU rDNA, or 18S rDNA) sequence analysis, and also has been supported with studies of elongation factor and three other proteins: alpha- and beta-tubulin and actin (Baldauf and Palmer, 1993; Bruns et al., 1991; Wainright et al., 1993). However, the hypothesis has been challenged by comparison of RNA polymerase gene sequences and reanalysis of SSU rDNA data (Rodrigo et al., 1994; Sidow and Thomas, 1994).

In the absence of many derived morphological (other than hyphal growth in some species) and biochemical characters, Chytridiomycota (previously characterized by the presence of a flagellate cell in the life cycle) now has been redefined by SSU rDNA sequence analysis. The study also has shown that some non-flagellate species traditionally placed in Zygomycota actually are chytrids (ex. Basidiobolus ranarum) (Nagahama et al., 1995).

Zygomycota as traditionally delimited on the basis of often non-septate hyphae and production of zygospores is not monophyletic. The position of some lineages such as that of Glomales and of Engodonales-Mortierellales is unclear, but they too may lie outside Zygomycota as independent lineages basal to the Ascomycota-Basidiomycota lineage (Bruns et al., 1993; K. O'Donnell, personal communication, 1996).

Evidence from shared morphological characters such as regularly septate hyphae and a dikaryotic stage (two separate and different nuclei in a single hyphal segment) in the life cycle, usually has been interpreted as support for a close relationship between Basidiomycota and Ascomycota. SSU rDNA adds support for this hypothesis (Berbee and Taylor, 1992).

Molecular characters have been essential for phylogenetic analysis in cases when morphological characters are convergent, reduced, or missing among the taxa considered. This is especially true of species that never reproduce sexually, because characters of sexual reproduction traditionally have been the basis for classification of Fungi. Use of molecular characters allows asexual fungi to be placed among their closest relatives. Excluded groups previously considered to be Fungi are stramenopiles (Oomycota, Hyphochytriomycota, and Labyrinthulomycota) and slime molds (Myxomycota, Plasmodiomycota, Dictyosteliomycota, Acrasiomycota) (Bhattacharya et al., 1992; Leipe et al., 1994; Van der Auwera et al., 1995).

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Information on the Internet

Popular Sites

- MykoWeb. WWW pages devoted to the science of mycology and the hobby of mushrooming.
- Introduction to the Fungi. UCMP Berkeley.
- Mycological Society of San Francisco. North America's largest local amateur mycological association.
- The Fungal Jungal. To further educate people about fungi, edible and otherwise, To encourage sustainable and responsible mushroom harvest, and preserve mushroom habitat.
- Tom Volk's Fungi.
- Dave Fischer's North American Mushroom Basics.
- MushroomExpert.Com.
- Forest Fungi.
- Pilze, Pilze, Pilze. In deutsch.
- Westfälischen Pilzbriefe. In deutsch.

- Micologi Associati. Nell'italiano.

Directories, Databases & Collections

- The WWW Virtual Library: Mycology. A well indexed entrance to almost all mycology and fungal biology resources on the Internet.
- Mycology.Net. An internet site containing information about diversity of fungi.
- Mycorrhiza Information Exchange.
- Mycology Online. A WWW resource of clinically significant mycological information.
- Yahoo Mycology.
- Mycologists Online. World-wide directory for mycology and lichenology.
- FungalWeb. A classification for all fungal genera based on a combination of phenotypic and genotypic data.
- Fungal Databases. Systematic Botany and Mycology Laboratory. Agricultural Research Service. United States Department of Agriculture. Beltsville, Maryland, USA.
- The Fifth Kingdom online. A mycological encyclopedia.
- Mycological and Lichenological Collection Catalogs. UCMP Berkeley.
- University of Alberta Microfungus Collection & Herbarium (UAMH).
- University of Michigan Fungus Collection.
- Mycological Herbarium. The Natural History Museums and Botanical Garden, University of Oslo.
- Herbarium Mycologicum. National Botanic Garden of Belgium.
- CABI Bioscience Database of Fungal Names (Funindex)
- Centraalbureau voor Schimmelcultures (CBS). Fungal Biodiversity Center - Utrecht, The Netherlands.
- Fungal Genetics Stock Center.
- Canadian Collection of Fungal Cultures.

Images

- Treasures from the Kingdom of Fungi. Taylor Lockwood's mushroom photography.
- Fungus plates painted under the supervision of Elias Fries. Department of Cryptogamic Botany, SwedishMuseumof NaturalHistory.
- Fungi Images on the Net.
- Fungi, Moulds and Lichens. BioImages: The Virtual Field-Guide (UK).
- Kinok-ya: Portraits of Mushrooms from Japan.
- Fungi in Finland and in Sweden.
- George Barron's Website on Mushrooms and other Fungi.
- Eileen's Mushroom Mania.
- Herrera's Microfungi Home Page.
- Nathan's Fungi Page.
- Pamela's Mushrooms.

Research Labs & Projects

- Deep Hypha. NSF-funded Research Coordination Network (RCN) that is focused on developing robust phylogenetic hypotheses for the deep branches within Kingdom Fungi and enhanced research and educational tools in fungal systematics.
- AFTOL: Assembling the Fungal Tree of Life. Collaborative research in fungal phylogenetics.
- Systematic Botany and Mycology Laboratory. Agricultural Research Service. United States Department of Agriculture. Beltsville, Maryland, USA.
- Forest Mycology and Mycorrhiza Research Team. Forestry Sciences Laboratory, Corvallis, OR, USA.
- Cornell Center for Fungal Biology (CCFB).
- Bruns Lab. University of California at Berkeley. Ecology and evolution of fungi.
- Spatafora Lab. Oregon State University. Systematics and evolutionary biology of fungi.
- Taylor Lab. University of California at Berkeley. Evolutionary relationships of fungi, concentrating on the fungi that cause human disease.
- Thorn Lab. University of Western Ontario. Fungal ecology and systematics.
- Vilgalys Lab. Duke University. Natural history of fungi, including all aspects of

their evolutionary biology, population genetics, and systematics.

- MycoSite. University of Oslo, Norway.
- University of Georgia Mycology.
- University of Tennessee Mycology Lab.
- Virginia Tech Mycology Lab.
- Fungal Mitochondrial Genome Project (FMGP). B. Franz Lang, Université de Montréal.
- Fungimap Australia. A collaborative project between professional and amateur mycologists and naturalists to gather information about the distribution of fungi throughout Australia.
- The Fungi of New Zealand. Manaaki Whenua Landcare Research.
- MycoKey. Thomas Læssøe & Jens H. Petersen, University of Aarhus provide:
  - Systematic Overview of the Kingdom Mycota
  - Synoptic key for determination of Northern European genera of fungi.
  - History of Mycology
  - MycoBase: records of Danish fungi
  - Fungi of Burkina Faso (West Africa)
- Mapping of Macromycetes in Norway. A co-operation between the Norwegian Mycological Society and the Norwegian Ethno-Botanical Society with their local associations and the Museums of Natural History in Oslo, Bergen, Trondheim and Tromsø.
- Comparative Studies on the Macrofungi of China and Eastern North America. Qiuxin Wu & Gregory M. Mueller, The Field Museum, Chicago.
- Survey of Northern Illinois and Indiana Fungi. John F. Murphy & Gregory M. Mueller. The Field Museum, Chicago.
- Macrofungi of Costa Rica. Roy E. Halling & Gregory M. Mueller.
- The Fungi of Kenya.
- Malawi Fungi.
- Chytrid Fungi Online. A PEET project website.
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Professional Societies

- The International Mycological Association. A group that represents mycologists and fungal biologists throughout the world.
- British Mycological Society.
- Mycological Society of America.
- Asociación Latinoamericana de Micología.
- Australasian Mycological Society.
- The International Society for Mushroom Science. To further the cultivation of edible (including medicinal) macrofungi.

Title Illustrations

Scientific Name Chytridium (Chytridiomycota)

Comments Individual growing on a single pine pollen grain. Successive photos show zoospore release from the sporangium, and the arrow points to a flagellum.

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Scientific Name Pilobolus (Zygomycota)

Comments Black sporangium atop swollen sporangiophore. Shortly, the swollen subsporangial vesicle will burst to send the sporangium flying. Herbivores eat the sporangium, and the enclosed mitospores germinate in the dung. The bright yellow carotenoid pigment enables the sporangium to orient to light (phototropism). If you look closely, you can see masses of nematodes on the vesicle; probably herbivore pathogens hoping to hitch a ride.

Specimen Condition Live Specimen

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Scientific Name Laetiporus sulphureus (Basidiomycota)

Comments Massed fruiting bodies of the chicken-of-the-woods. The tiny tubular filaments (hyphae) that make the body of this fungus (mycelium) are growing in the old, dead wood of a large cherry tree. Laetiporus is not a parasite, but the decay may weaken the tree so much that wind or ice storms can topple it.

Specimen Condition Live Specimen

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Scientific Name Scarcoscypha coccinea (Ascomycota)

Comments Fruiting body of the scarlet cup fungus. Hundreds of millions of meiospores (ascospores) are discharged from this cup, usually in puffs that produce visible clouds of spores

Copyright © 1996 Joey Spatafora

About This Page

Many thanks to Soren Rosendahl and Atul Batra for scanning photos and David Maddison and Atul Batra for page design advice.

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Content changed 14 February 2005

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to that?
A. Because she just kept directing the conversation back to the fact that she thought all of her symptoms were due to exposure to fungus and molds and didn't want to hear what I was -- she seemed not to want to hear what I had to say, and I tried to convince her that she could be better if she would take medication and stop -- continue to not smoke.
Q. Over the almost two years that you saw and treated Ms. Jazairi, had you considered mold exposure as a potential cause of her symptoms?
A. Yes.
Q. And what had you concluded in regard to that?
A. Well, I think that when I first saw her, it's possible that she could have had a problem from the mold. It's possible.
But having moved out of the environment and as time passed, it didn't appear to be so, at least as far as I could tell.
Q. If I could have you take a look back at Exhibit 65, Impression Number 1, if you could just read that into the record for me.
A. What is it that you want me to read?
Q. The impression discussion, Number 1.

00:55:41-00:56:34 Page 42

A. On July 20th?
MR. THOMPSON: Yes.
MR. BROOKS: Objection to form.
THE WITNESS: "Impression, Number 1, I see no reason to blame any other disease process other than asthmatic bronchitis due to cigarette smoking for the chest symptoms in the patient. I do not understand why she is focusing so much on this fungal exposure. I cannot imagine what fungal disease would linger and cause all of the symptoms she is complaining of. It is not clear that she ever had interstitial lung disease, although initially her chest x-ray was suggestive of it. And, two, tobacco addiction."
Now, when I write my impression, I'm generally dealing just with the pulmonary issues here. I'm not going over any other things that she had.
Q. (By Mr. Thompson) And was that what you just read, was that your impression as of July 20th, 2004?
A. It was.
MR. BROOKS: Object to the form.
Q. (By Mr. Thompson) And have you reached that opinion based on a reasonable degree of medical

00:56:35-00:57:43 Page

certainty?
A. Yes.
Q. And if you could just describe for me generally how you reached that opinion.
A. She was wheezing, coughing. She had mucus. Her chest x-ray had gotten better. There was no indication on the pulmonary function test of an interstitial lung problem, which is usually manifested with low lung volumes and sometimes diffusion abnormalities.
It all fits. This is what I see -- 80 percent of what I see in my practice is asthmatic bronchitis, and I see it over and over and over again.
Q. And you've been practicing close to 25 years?
A. Correct.
Q. You also note tobacco addiction. What did you mean by that?
A. She had only been off cigarettes -- what she told me in the history is that she had only been off cigarettes for a short time and she was using a Nicotrol inhaler.
So certainly there's always a chance that someone will go back to cigarettes and I wouldn't

00:57:46-00 58:57 Page 4

have considered the addiction gone. It's too soon.
Q. And you also after your impression discussion you have a plan of management. What does that mean?
A. It was my suggestion for that day, that visit.
Q. What was your first suggestion?
A. Continued encouragement in regard to smoking cessation.
Q. And did you provide that guidance to Ms. Jazairi?
A. Yes.
Q. Then what was your second plan of management?
A. "Treat symptoms by starting Spiriva and Advair. I have sincere doubts whether she is going to take this medication because I believe she wants to continue having symptoms to try to make a case for this fungal infection."
MR. BROOKS: Objection, relevance.
Q. (By Mr. Thompson) And did you -- what did you mean by that?
A. Because she kept trying to direct all of her symptoms back to the fungus and she seemed to be almost unhappy when I suggest

EXHIBIT tabbies 10

IN THE UNITED STATES DISTRICT COURT
FOR THE SOUTHERN DISTRICT OF GEORGIA
SAVANNAH DIVISION

CHRIS JAZAIRI, Plaintiff. VS. ROYAL OAKS APARTMENT ASSOCIATES, L.P., Its Parent Company And Subsidiaries, And MITCHELL L. MORGAN MANAGEMENT, INC., Defendants.	)	CIVIL ACTION FILE NO.: CV-04-404-091

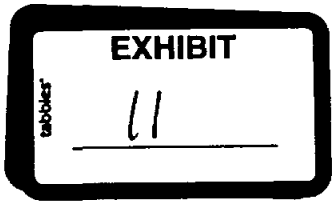
STATE OF GEORGIA)
)
COUNTY OF _____)

AFFIDAVIT OF W. ELLIOTT HORNER, PH.D.

1. Affiant is of legal age and gives this affidavit based on personal and professional knowledge with the understanding that this affidavit will be filed on Plaintiff's behalf in the pending action of *Chris Jazairi v Morgan Mitchell Properties, et al.*, U.S. District Court, Southern District of Georgia, Savannah Division, Civil Action No. CV-04-404-091.

2. Affiant is a mycologist and works with Air Quality Sciences [AQS] in Atlanta, Georgia. Affiant's educational background includes a MS in Mycology from the State University of New York, College of Environmental Science and Forestry and a PhD in Forest Pathology from VPI & SU. Affiant also undertook a post-doctoral fellowship at Tulane University School of Medicine conducting laboratory research on fungal allergens. The field of mycology includes the study of molds and other fungi, including fungi causing root diseases of trees.

3. Affiant's work with AQS often involves analysis of samples of building material



to determine whether such materials have been colonized by fungi (have mold growth). Affiant's work also involves analysis of environmental samples taken from buildings for the purpose of detecting indications of fungal colonization (mold growth) in the building. Affiant also conducts evaluations that artificially expose building materials to mold to determine the resistance of these material to mold growth. AQS performs environmental assessments and advises clients, including many corporate clients, on the need for and appropriate methods for remediation of mold colonized (moldy) material indoors that can generate mold contamination. The microbial laboratory at AQS is accredited through the American Industrial Hygiene Association's laboratory accreditation program for environmental microbiology (EMLAP). The laboratory uses well accepted procedures in the analysis of environment samples that it receives, follows written procedures for these analyses and has a formal, written quality system in place that is compliant with ISO 17025 (an international quality system standard for testing laboratories).

4. Affiant is familiar with molds and fungi in his work and has published in peer-reviewed scholarly journals in the field, as well as presented at numerous conferences in the field. Affiant's curriculum vitae is attached as Exhibit A to this affidavit. Affiant issued the lab report on the samples sent from the Chatham County Department of Health [CCDH], sample nos.09586-010AAz, BF1m and nos.09586-010AAz, BF2m. These samples were received on September 5, 2002 in appropriate sample containers that were appropriately marked. The transfer of dry bulk samples by mail to AQS is acceptable.

5. Affiant understands that the bulk mold samples were collected by the CCDH on or about August 2, 2002. If the samples were dry at the time of collection, the one month interim between collection of the samples and analysis of the samples is not of any significant scientific consequence if the purpose of the analysis is simply to determine what molds were present in the

environment where the molds were collected. If the dry samples were stored in clean, dry and intact plastic bags at room temperature, then the molds present in the bags would not have grown, i.e. there would be no growth. Furthermore, any fungi detected on bulk samples that were stored in intact, sealed containers would be derived from the sample at the time of collection (or the environment in which the container was sealed). The methodology of the sample collection, storage and transfer, as reported to affiant are acceptable. The analysis complied with commonly accepted scientific methods for identification of molds in a living environment. The analysis of the samples was completed on September 20, 2002.

6. The samples received from the CCDH were analyzed for molds using routine dilution plating methods, which is a well accepted methodology, a copy of which is attached as Exhibit B. The report is attached as Exhibit C. This report is of scientific value for determining the types of molds present on the sample and/or in the environment where the samples were taken. Different molds have different properties and have different growing characteristics. For instance, Stachybotrys chartarum often colonizes materials rich in amorphous cellulose (paper) that are very wet (not just damp) either from prolonged wet conditions or from being wetted repeatedly. Stachybotrys chartarum was found in both of the samples analyzed. Wallemia sebi however thrives under conditions that are far drier, and hence often grows in dust indoors. Dry tolerant molds were absent from the samples.

7. The molds reported in the analysis to the CCDPH in Exhibit C included Stachybotrys chartarum, Aspergillus niveus, Aspergillus puniceus, Aspergillus versicolor. These molds are not usually present as visible growth in an indoor environment, but are not unusual as components of visible mold on water damaged building materials. The presence of visible mold (fungal colonization), as reported to affiant, in an indoor environment should create

concern for an atypical exposure of humans to spores and other fungal-derived particles from surfaces with visible mold.

8. Molds release spores and other particles into the air at variable rates. The release rates can depend on the life cycle of the particular mold, the environmental conditions at a given time, and likely also the degree of degradation of the substrate (e.g superficially colonized ceramic tile vs. badly rotted wallboard). Different mold spores have different biological properties and can be expected to cause different reactions in humans. Mold growth produces multiple substances (metabolites) that are biologically active, such as glucans, allergens, proteases, and in some cases toxins. Particles with biological activity other than intact spores (broken spores, fungal fragments and substrate particles with mold metabolites) are also released from moldy surfaces. A recent research paper from a peer-reviewed journal demonstrating this is attached as Exhibit D. Due to the large number of different molds, and the variety of substances from each, there are inadequate resources to test all of the molds in all the possible combinations to determine all possible effects on humans. However, it is known that mold spores and mold substances can cause reactions in humans. Affiant has published articles in peer-reviewed journals on various substances released by molds that can cause reactions by humans. These publications include book chapters and peer reviewed articles including the following:

1. Morey, PR, Horner WE, Epstien BL, Worthan AG and Black MS, "Indoor Air Quality in Nonindustrial Occupational Environments", in R.L. Harris (Ed.) Patty's Industrial Hygiene (5th ed. pp 3149-3241). John Wiley & Sons, NY (2000);

2. Horner, WE, Lehrer, SB and Salvaggio, JE, "Aeorbiology" (pp 53-72) in RF Lockey, and SC Burkartz, Allergens and Allergy Immunology (2d ed) Marcell

Dekker, NY(1999);

3. A. De Zubiria, W. E. Horner and S. B. Lehrer. 1990. Evidence for cross-reactive allergens among basidiomycetes: immunoprint-inhibition studies. J. Allergy Clin. Immunol. 86:26-33

4. S. Wongtim, J. E. Salvaggio, S. B. Lehrer and W. E. Horner. 1993. Protease activity in cockroach and basidiomycete allergen extracts. Allergy Proc. 14:263-268.

5. W. E. Horner, E. Levetin and S. B. Lehrer. 1993. Basidiospore allergen release: elution from intact spores J. Allergy Clin. Immunol. 92:306-312.

6. W. E. Horner, J. M. Hughes, M. Lopez and S. B. Lehrer. 2000. Basidiomycete skin test reactivity is a risk factor for asthma. Ped. Asthma, Allergy Immunol. 14:69-74.

7. W.E. Horner, A.G. Worthan, P.R. Morey. 2004. The Air and Dustborne Mycoflora in Houses Free of Water Damage and Fungal Growth. Applied and Environmental Microbiology. 70:6394-6400.

9. Air sampling for spores or culturable particles is not always reliable for determining the amount of mold exposure in an environment. Air sampling generally relies on the recovery of intact spores for identification by microscopy, or the recovery of a culturable particle. As stated above, this only accounts for a portion of the material that is released from moldy surfaces. Further, release of mold spores is variable, so even spore-based analyses are inherently susceptible to error, unless sampling is repeated multiple times. Air tests taken within

an hour of each other can provide very different results. Present methodology for investigation of the extent of mold and fungus contamination in a living or work environment emphasizes observation of the square footage of visible mold (and to the extent possible, including visible mold in hidden spaces, such as wall voids) in an environment as a surrogate marker for exposure. The square footage as surrogate for exposure is used by both the EPA guidelines and the New York City guidelines in determining what precautions are prudent for conducting mold remediation. The EPA and New York City mold remediation guidelines are well accepted in the mold remediation field. Additionally, the attached articles note that visible indicators of water damage (such as visible mold) can identify damp indoor environments, and as such are a surrogate for determining whether an atypical mold exposure is present in an indoor environment Exhibits E, F. The identification of damp indoor spaces is crucial since these favor mold growth and both damp indoor spaces and mold growth have been identified in a recent report from the Institute of Medicine as associated with deleterious human health effects.

10. These remediation guidelines mentioned above recommend some form of containment when amounts over 10 square feet of mold-colonized material is removed. The purpose of the containment is to prevent the contamination of other areas of the living or working environment by the mold spores, and to limit exposure of remediation workers to mold dusts and spores. When moldy materials are disturbed, spores and other mold-related particles are released, often in massive numbers, that can travel in the air. For instance, if mold-colonized gypsum wallboard in one room of a house is removed with a saw, hammer or crowbar, massive numbers of particles are released. Large numbers of these particles are small enough to be breathed into the lung. These particles (including spores) can travel into other rooms and accumulate as dust and if spores contact moisture, they can colonize previously non-moldy areas.

Humans in these other rooms can also be exposed to the traveling airborne mold spores and particles, including mold spores and particles that were previously not present for inhalation by humans in those areas.

AND FURTHER AFFIANT SAYETH NOT.

W. Elliott Horner

ELLIOTT HORNER, PH.D.

Sworn to and subscribed

before me this 7 day

of March, 2005.

Kim A Munroe

Notary Public,

Cobb County, Georgia

(NOTARIAL SEAL)

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CURRICULUM VITAE

W. Elliott Horner, PhD, FAAAAI

Air Quality Sciences, Inc.
1337 Capital Circle, Atlanta, GA, 30067
(770) 933-0638 Ehorner@aqs.com

Education:

Ph.D. 1985. (Forest Pathology) Virginia Polytechnic Institute and State University. Department of Plant Pathology, Physiology and Weed Science.

M.S. 1981. (Mycology/Plant Pathology) State University of New York, College of Environmental Science and Forestry. Department of Biology, Chemistry and Ecology.

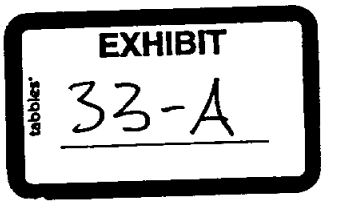
B.A. 1976. (Botany and Comparative Literature) University of North Carolina at Chapel Hill, College of Arts and Sciences.

Appointments/Employment:

Laboratory Director, Microbiology. 1996-present, Air Quality Sciences, Inc., Atlanta, GA.
Adjunct Research Assistant Professor of Medicine. 1996-present, Tulane Medical Center, New Orleans, LA.
Adjunct Assistant Professor of Environmental Health Sciences, 1995-present, Tulane University School of Public Health and Tropical Medicine, New Orleans, LA
Research Assistant Professor of Medicine. 1991-1996, Tulane Medical Center, New Orleans, LA.
Postdoctoral Research Fellow. 1986-1990, Tulane Medical Center (Dr. Sam Lehrer, supervisor), New Orleans, LA.
Graduate Research Assistant. June, 1981-December, 1985, VPI&SU (Dr. Sam Alexander, advisor), Blacksburg, VA.
Graduate Research Assistant. July, 1979-June, 1981, College of Forestry (Dr. Robert Zabel, advisor), Syracuse, NY.
Herbarium Assistant. September, 1978-June, 1979, N.C. State U. (Dr. James Hardin, supervisor), Raleigh, N.C.
Naturalist and CETA Supervisor. January, 1977-July, 1978, Weymouth Woods-Sandhills Nature Preserve (NC State Parks), Southern Pines, NC

Professional Affiliations:

American Academy of Allergy and Immunology
American Industrial Hygiene Association
American Society for Microbiology
Mycological Society of America
Pan American Aerobiology Assn
ASHRAE



University Service:

Tulane/LSU Clinical Immunology Seminars: Coordinated the 1990-1991 series and the 1991-1992 series.

Organized a mini-symposium of current molecular biology techniques as part of the 1990-1991 series.

Professional Society Service:

American Industrial Hygiene Association, Biosafety Committee, Laboratory Quality Assurance Program (LQAP) EMLAC Committee 2000-2003, Chair 2002; LQAP Analytical Accreditation Board 2002-2003; LQAP Technical Advisory Panel 2004

American Academy of Allergy, Asthma and Immunology, Aerobiology Committee, Allergen Standardization Committee, Indoor Allergen Committee (Vice Chair 2002), Air Pollution Committee

Mycological Society of America, Environmental Health and Medical Mycology Committee 1996-1998, Chair 1998

Grants Received and Contracts Awarded:

HUD: Contract Awarded to Air Quality Sciences, Inc. Co-Principal Investigator, W. E. Horner. HUD Healthy Homes Research Program
Project Period: January 2002 – July 2003

ASHRAE: Contract Awarded to Air Quality Sciences, Inc. Co-Principal Investigator, W. E. Horner. Detection and Removal of Gaseous Effluents and Byproducts of Fungal Growth that Affect Indoor Environments
Project Period: January 1999- October 1999

Center for Indoor Air Research: Contract Awarded to Air Quality Sciences, Inc. Principal Investigator, W. E. Horner. Microbial VOCs as Indicators of Indoor Fungal Growth and Sources of Irritation
Project Period: January 1997- September 1999

Center for Indoor Air Research: Contract Awarded to Tulane Clinical Immunology Section, Principal Investigator, W. E. Horner. "Quantitative Assessment of Indoor Fungi: Immunologic and Biochemical Methods"
Project Period: January 1996-December 1998

Center Laboratories, Port Washington, NY: Contract awarded to Tulane Clinical Immunology Section, Co-Principal Investigator, W. E. Horner. "Acarasan Comparative Efficacy Study"
Project Period: April 1996-October 1997

Louisiana Department of Natural Resources: Cooperative Agreement Awarded to Tulane Clinical Immunology Section, Principal Investigator, W. E. Horner. "Indoor Air Quality in Louisiana Residences"
Project Period: October 1994-September 1996

Louisiana Department of Environmental Quality: Contract Awarded to Tulane Clinical Immunology Section, Principal Investigator, W. E. Horner. "Detection of Airborne Grain Antigen in St. Charles Parish, LA"
Project period: March 1994-February 1995

Grants Received and Contracts Awarded: (con't.)

Louisiana Department of Environmental Quality: Contract Awarded to Tulane Clinical Immunology Section, Project Coordinator, W. E. Horner. "Aeroallergen Monitoring Program for Baton Rouge, LA"
Project period: May 1990-June 1993

American Lung Association Research Grant: Principal Investigator, W. E. Horner. "Basidiomycete Allergen Analysis Using Monoclonal Antibodies"
Project period: July 1991-June 1993

Hyde and Watson Foundation Research Grant: Co-investigators, J.E.Salvaggio and W. E. Horner. "Mechanism of action of basidiospore allergens in asthma"
Project period: May 1990-April 1991

NIH Small Instrumentation Grant (to Tulane Medical Center) 1991-1992: Awarded $7,500 for electrophoresis equipment in university competition.

Awards:

Voted Fellow of the American Academy of Allergy, Asthma and Immunology, 2002

Young Faculty Award: 1994, 1995
American Federation for Clinical Research (Southern Section)

Competitive Travel Grants:
VII International Congress of Immunology at Berlin, August, 1989, (awarded by the American Association of Immunologists)
International Congress of Allergology and Clinical Immunology (XIII ICACI) at Montreux, Switzerland, October, 1988, (awarded by the Congress)

Musser-Burch Society (honorary society): 1987
Tulane University Department of Medicine

Arthur J. Webber Award (outstanding graduate student): 1984
Department of Plant Pathology, VPI&SU

Teaching Activities and Invited Lectures:

American Academy of Allergy, Asthma and Immunology, 2004 Evaluation of Homes and Buildings with Mold-Related Problems in the workshop "Evaluation of Mold-Related Health Problems" 22 March, 2004 (San Francisco)

American College of Allergy, Asthma and Immunology, 2003. Environmental Assessment for Mold in workshop 7 November 2003 (New Orleans)

Mycological Society of America / British Mycological Society, 2003. "Standardization of Lab Methods / Accreditation" in the "Expert Indoor Mycology" workshop, joint meeting 31 July 2003

Louisiana Society of Allergy, Asthma and Immunology 2003. "The Biology of Molds" (28 June 2003) and "Environmental Analysis Methods and Interpretation to Assess Indoor Exposure to Fungi, Spores and Fungal Toxins" (29 June 2003) Annual Meeting (New Orleans)

American Society For Microbiology, 2003. 103rd General Meeting. "Enhanced MVOC Detection for Monitoring Building Air for Mold Biocontamination." In the colloquium "Fungal Biocontaminants In Indoor Environments." 20 May, 2003 (DC).

Teaching Activities and Invited Lectures: (con't).

Michigan Safety Conference, 2003. Invited speaker for Industrial Hygiene Division "Mold Remediation Case Studies." 15 April, 2003 (Lansing, MI).

Healthy Indoor Environments 2003 Conference Proceedings. Invited speaker for Healthy Indoor Environments seminar "Comparative Sampling Techniques for Mold Investigations." 9 April, 2003 (Anaheim, CA).

American Academy of Allergy, Asthma and Immunology, 2003. 60th Anniversary Meeting. "Environmental Analysis Methods and Interpretation to Assess Indoor Exposure to Fungi, Spores and Fungal Toxins." In the AAAAI Postgraduate Symposium "Health Effects of Indoor Molds: The Known, the Suspect and the Unproven." 9 March, 2003 (Denver).

American College of Allergy, Asthma and Immunology, 2002 "Biology of Molds" Plenary Session, Annual Meeting, 16 November, 2002 (San Antonio)

American Industrial Hygiene Conference and Exhibition, 2002 "Strengths and Limitations of Microbial Sampling and Analytical Methods" in the AIHCE Forum "Mold Investigations: The current state of knowledge" 3 June, 2002 (San Diego)

American Academy of Allergy, Asthma and Immunology Annual Meeting. 2002 Mold and Mycotoxins: Exposure Assessment and Health Risks. (Breakfast Seminar) 5 March 2002 (New York)

American Academy of Allergy, Asthma and Immunology, 2002. Moderator and Presentation of "Environmental Laboratory Testing for the Environment: Constraints and Clues" in the AAAAI Workshop "Handling Distressed Patients Claiming Environmental Disease" 4 March, 2002 (New York)

American Academy of Allergy, Asthma and Immunology, 2002. "Exposure Assessment and Interpretation of Results" in the AAAAI Symposium "The Allergist and Indoor Air Quality: How to Handle Building Related Illnesses" 3 March, 2002 (New York)

American Academy of Allergy, Asthma and Immunology, 2002. "Sampling for Indoor Molds and Interpreting the Results" in the AAAAI Workshop "Building Inspections and the Allergist" 3 March, 2002 (New York)

American Academy of Allergy, Asthma and Immunology, 2002 - 2004. "Sampling and Analysis for Indoor Mold" in the Advanced Aeroallergen Course" 2 March, 2002 (New York), 8 March 2003 (Denver), 22 March 2004 (San Francisco)

American Academy of Allergy, Asthma and Immunology, 2002 - 2004. "Nature of Outdoor Fungus Spore Populations" in the "Basic Aeroallergen Course" 1 March, 2002 (New York), 7 March, 2003 (Denver), 19 March, 2004 (San Francisco)

American Public Health Association. 2001. Biological / Microbial Samples from Buildings: How are they handled and what can they tell us? in the CEI course "Air Quality Problems in Public Buildings – Assessment, Control and Prevention" 21 October, 2001 (Atlanta)

Aerias Annual National Symposium. 2001. Indoor Molds: Widely Used, New and Experimental Sampling Methods and Interpretation. 19 October, 2001 (Atlanta)

Teaching Activities and Invited Lectures: (con't).

National Environmental Health Association. 2001. Allergens, Molds and endotoxins: Do they link to the asthma epidemic? 1 July, 2001 (Atlanta)

American Institute for Conservation of Historic and Artistic Works. 2000 and 2001. Postconference Workshop on Practical Aspects of Mold Remediation for Cultural Property. 13 June 2000 (Philadelphia) and 4 June 2001 (Dallas)

American Industrial Hygiene Conference and Exposition. 2000. Microbial VOCs (MVOCs) as an effective IEQ Screening Tool. Invited presentation in the AIHA Roundtable on Indoor Volatile Organic Compounds from Chemical and Microbial Sources. 22 May 2000

University of Tulsa / USEPA Workshops. 2000. Indoor Air Quality: Asthma and Allergen Control. Denver 3/9/00; Atlanta 4/28/00; San Diego 9/12/00

American Academy of Allergy, Asthma and Immunology Annual Meeting. 2000, 2001, 2002, 2003. Sick Building Syndrome: Clinical and Environmental Assessment. (Lunch Seminar) 4 March 2000 (San Diego), 17 March 2001 (New Orleans), and (Breakfast Seminar) 6 March 2002 (New York), 9 March, 2003 (Denver)

Clemson University Extension Service. Healthy Indoor Air in Warm Humid Climates Inservice Training Course). Environmental Assessment - Limits and Uses of Sampling, and Current Research and Future Needs. 19 January, 2000

American Industrial Hygiene Conference and Exposition. 1999, 2000, 2001, 2002. Solving Indoor Air Quality Problems in Hot/Humid Climates. Professional Development Course, 5 June, 1999 (Toronto), 20 May, 2000 (Orlando), 2 June, 2001 (New Orleans), 1 June, 2002 (San Diego)

Third International Conference on Bioaerosols, Fungi, and Mycotoxins. 1998. Why are There Still Problems with Fungal Extracts? Saratoga Springs, New York 23-25 September, 1998

American Industrial Hygiene Conference and Exposition. 1998. Toxigenic Molds as Allergens. Are toxic fungi special allergens? Invited presentation in the AIHA Roundtable on Toxigenic Molds. What is the Evidence? 14 May 1998

MidAltlantic Environmental Hygiene resource Center. 1998. Fungi and Microbial VOCs in Indoor Air. What does the Data Mean? How much mold is too much? Invited Presentation in the Symposium on Microbiological Contamination of Indoor Environments. New Orleans 4-6 February 1998

University of Tulsa / USEPA Workshops. 1997 - 1999. Sampling for Biological Contamination in Buildings: Methods, considerations and implications. Atlanta, 11/20/98; Walnut Creek, CA 12/12/97; West Palm Beach, FL 4/23/98; Dallas 5/21/98; Los Angeles 6/17/98, Honolulu 5/20/99, Denver 6/25/99

Mycological Society of America. 1997. Aerobiology, The Real World. Invited presentation in the Symposium on Fungi and Sick Buildings. Montreal 3-7 August, 1997

Society for Industrial Microbiology. 1997. Detection of Early and/or Hidden Microbial Contamination by Microbial Volatile Organic Compound (MVOC) Analysis. Invited presentation in the Symposium on Microorganisms and Indoor Environmental Quality. Reno 2-3 August, 1997

Teaching Activities and Invited Lectures: (*con't*).

MidAtlantic Environmental Hygiene resource Center. 1997. Microbial VOCs: sampling, potential health effects and indoor microbial contamination. Invited Presentation in the Symposium on Biological Contamination of Indoor Environments. Chicago 16-18 April 1997

American Society for Microbiology, 1996. Health Effects of Exposure to Microbial Agents in Indoor Environments. Invited Presentation in Workshop: Indoor Air Quality. 95th ASM General Meeting, New Orleans 19-23 May 1996.

American Academy of Allergy, Asthma and Immunology, 1996. Nature of Outdoor Fungus Spore Populations. Invited Presentation in the Aeroallergen Identification Workshop. 52nd Annual Meeting, New Orleans 15-20 March 1996.

American Society for Microbiology, 1994. Immunoassays for Fungal Bioaerosols: Prospects and Problems. Invited presentation at Round Table: Progress in methods development for monitoring microorganisms in bioaerosols. 94th ASM General Meeting, Las Vegas 24-27 May 1994.

Tulane University School of Public Health and Tropical Medicine. 1993-1996. Served on thesis committee for two Ph.D. candidates.

Tulane Medical Center. 1986, 1988, 1991, 1993. Provided guidance and laboratory instruction for two medical students and three clinical fellows for their research projects. (Manuscript coauthor with each fellow).

Department of Plant Pathology, Physiology and Weed Science, VPI&SU. 1982, 1983. Planned and coordinated for two years the undergraduate laboratory for Forest Pathology (Dr. Alexander). Designed laboratory exercises and compiled a laboratory guide. Presented lectures in the classroom covering fungal decay of wood and wood products.

Botany and Plant Pathology section of the Department of Biology, Chemistry and Ecology, SUNY College of Forestry. 1981. Assisted with the undergraduate laboratory for Forest Pathology (Dr. Paul Manion).

Manuscript Reviewer for:

Aerobiologia
Aerosol Science and Technology
Allergy
American Industrial Hygiene Association Journal
Canadian Journal of Botany
Clinical and Experimental Allergy
Environmental Research
International Archives of Allergy and Immunology
Journal of Allergy and Clinical Immunology

PUBLICATIONS

Thesis
W. E. Horner. Studies of Aureobasidium pullulans (de Bary) Arnaud on Latex Paint. 1981.

Dissertation
W. E. Horner. Etiologic Studies of Verticicladiella procera in Pine Christmas Trees. 1985.

Journal Articles
1. R. A. Zabel and W. E. Horner. 1981. An accelerated Laboratory Procedure for Growing Aureobasidium pullulans on Fresh Latex Paint Films. J. Coatings Technology. 53(675):33-37.
2. W. E. Horner, S. A. Alexander and M. M. Julian. 1986. Qualitative Determination of Cellulose in the cell walls of Verticicladiella procera. Mycologia. 78:300-303.
3. K. J. Lewis, S. A. Alexander, and W. E. Horner. 1987. Distribution and efficacy of propagules of Verticicladiella procera in soil. Phytopathology. 77:552-556.
4. W. E. Horner, S. A. Alexander, and K. J. Lewis. 1987. Colonization patterns of Verticicladiella procera in Scots and eastern white pine and associated resin-soaking, reduced sapwood moisture content and reduced needle water potential. Phytopathology. 77:557-560.
5. W. E. Davis, W. E. Horner, J. E. Salvaggio, and S. B. Lehrer. 1988. Basidiospore allergens: analysis of Coprinus quadrifidus spore, cap and stalk extracts. Clinical Allergy. 18:261-267.
6. M. D. Ibañez, W. E. Horner, V. Liengswangwong, J. Sastre and S. B. Lehrer. 1988. Identification and analysis of basidiospore allergens from puffballs. J. Allergy Clin Immunol. 82:787-795.
7. W. E. Horner, M. D. Ibañez, V. Liengswangwong, J. E. Salvaggio and S. B. Lehrer. 1988. Characterization of allergens from spores of the oyster mushroom, Pleurotus ostreatus. J. Allergy Clin. Immunol. 82:978-986.
8. W. E. Horner, M. D. Ibañez and S. B. Lehrer. 1989. Immunoprint analysis of Calvatia cyathiformis allergens: I. Reactivity with individual sera. J. Allergy Clin. Immunol. 83:784-792.
9. W. E. Horner, M. D. Ibañez and S. B. Lehrer. 1989. Stability studies of Calvatia cyathiformis basidiospore allergens. Int. Archs. Allergy Appl. Immunol. 90:174-181.
10. A. De Zubiria, W. E. Horner and S. B. Lehrer. 1990. Evidence for cross-reactive allergens among basidiomycetes: immunoprint-inhibition studies. J. Allergy Clin. Immunol. 86:26-33
11. C. E. O'Neil, W. E. Horner, M. A. Reed, M. Lopez and S. B. Lehrer. 1990. Evaluation of basidiomycete and deuteromycete (Fungi Imperfecti) extracts for shared allergenic determinants. Clin. Exp. Allergy 20:533-538.

Journal Articles (con't.)

12. R. P. Stankus, W. E. Horner and S. B. Lehrer. 1990. Identification and characterization of important cockroach allergens. J. Allergy Clin. Immunol. 86:781-787.
13. S. B. Lehrer, W. E. Horner, P. Menon and R. P. Stankus. 1991. Comparison of cockroach allergenic activity in whole body and fecal extracts. J. Allergy Clin. Immunol. 87:574-580.
14. W. E. Horner, J. Kailas, R. P. Stankus and S. B. Lehrer. 1990. Common German cockroach whole body and fecal allergens: immunoprint inhibition studies. Int. Archs. Allergy Appl. Immunol. 93:256-262.
15. C. E. O'Neil, M. A. Reed, W. E. Horner and S. B. Lehrer. 1991. Quantitation of specific IgG, IgA and IgM antibodies directed against four species of basidiomycetes. Grana. 30:142-146.
16. W. E. Horner, M. Lopez, J. E. Salvaggio and S. B. Lehrer. 1991. Basidiomycete allergy: identification and characterization of an important allergen from Calvatia cyathiformis. Int. Arch. Allergy Appl. Immunol. 94:359-361.
17. S. B. Lehrer, W. E. Horner, P. K. Menon, J. Oliver and P. Hauck. 1991. Cockroach allergenic activity: analysis of commercial cockroach and dust extracts. J. Allergy Clin. Immunol. 88:895-901.
18. E. Levetin, W. E. Horner and S. B. Lehrer. 1992. Morphology and allergenic properties of basidiospores from four Calvatia species. Mycologia 84:759-767.
19. A. Helbling, W. E. Horner and S. B. Lehrer. 1993. Identification of Psilocybe cubensis spore allergens by immunoprinting. Int. Archs. Allergy Immunol. 100:263-267.
20. W. E. Horner, A. Helbling and S. B. Lehrer. 1993. Basidiomycete allergens: Comparison of three Ganoderma species. Allergy 48:110-116.
21. A. Helbling, W. E. Horner and S. B. Lehrer. 1993. Comparison of Psilocybe cubensis spore and mycelium allergens. J. Allergy Clin. Immunol. 91:1059-1066.
22. S. Wongtim, J. E. Salvaggio, S. B. Lehrer and W. E. Horner. 1993. Protease activity in cockroach and basidiomycete allergen extracts. Allergy Proc. 14:263-268.
23. W. E. Horner, E. Levetin and S. B. Lehrer. 1993. Basidiospore allergen release: elution from intact spores J. Allergy Clin. Immunol. 92:306-312.
24. A. Helbling, G. Reese, W. E. Horner and S. B. Lehrer. 1994. Aktuelles zur Pilzsporen-Allergie. [Current aspects of fungal spores allergy] Schweiz Med Wochenschr 124:885-892.
25. J. J. Musmand, W. E. Horner, M. Lopez, S. B. Lehrer. 1995. Identification of important allergens in German cockroach extracts by sodium dodecylsulfate--polyacrylamide gel electrophoresis and Western blot analysis. J. Allergy Clin. Immunol. 95:877-885.

Journal Articles (con't.)

26. W. E. Horner, G. Reese and S. B. Lehrer. 1995. Identification of the allergen Psi c 2 from the basidiomycete Psilocybe cubensis as a fungal cyclophilin. Int Arch Allergy Immunol 107:298-300.
27. G. Reese, W. E. Horner and S. B. Lehrer. Characterization of basidiomycete allergens with monoclonal antibodies raised against Psilocybe cubensis spore extract. (submitted in consideration of publication; Int. Arch. Allergy Immunol.).
28. R. Lemus, A. A. Abdelghani, T. G. Akers and W. E. Horner. 1996. Health risks from exposure to metals in household dusts. Rev. Environ. Health 11:179-189.
29. R. Lemus, A. A. Abdelghani, T. G. Akers and W. E. Horner. 1997. Potential health risks from indoor exposure to chlorpyrifos (O-O-diethyl-O-[3,5,6-trichloro-2-pyridyl] phosphorothioate). Rev. Environ. Health 12:91-97.
30. R. Lemus, A. A. Abdelghani, T. G. Akers and W. E. Horner. 1998. Potential health risks from exposure to indoor formaldehyde. Rev. Environ. Health. 13:91-98.
31. W. E. Horner, J. M. Hughes, M. Lopez and S. B. Lehrer. 2000. Basidiomycete skin test reactivity is a risk factor for asthma. Ped. Asthma, Allergy Immunol. 14:69-74.
32. W.E. Horner, A.G. Worthan, P.R. Morey. 2004. The Air and Dustborne Mycoflora in Houses Free of Water and Fungal Growth. Applied and Environmental Microbiology. 70:6394-6400.

Conference Proceedings

W. E. Horner, G. Reese, and S. B. Lehrer. 1995. Field Assessment of an Immunochemical Assay for basidiomycete Fungal Antigens in Indoor Dust. pp 639-647. in Engineering Solutions to Indoor Air Quality Problems (Proc of a conference 24-26 July, 1995): Air and Waste Management Assn: Pittsburgh, PA

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revised date 6/22/04

AIR QUALITY SCIENCES
Building Consulting, Inc.

Summary of Method for Plating of Bulk, Swab and Dust Samples

- Dust and Bulk materials are weighed to determine total weight. The surface area of bulk samples is measured in cm and recorded. Apparent mold portions are selected, excised, weighed and a suspension in peptone tween is prepared.
- Dust is sieved to remove coarse particles and to mix the sieved materials.
- Platings are performed in replicate.

Direct Plating

- The weighed particles of dust are sprinkled onto the selected agar surface in replicate.
- Excised portions of bulk particles are placed onto the agar surface so as not to overlap each other.

Dilution Plating

- Suspend enough material in the diluent to create a 10^{-1} mixture
- 0.1g (dust), 1.0g (bulk), are suspended in peptone/tween [1:10 /W:V 0.9mL (dust), 9 mL (bulk)]. Smaller weights may dictate alternate volumes.
- The suspensions are mixed on a rotary shaker for 2 minutes, or for smaller volumes 1 minute on the vortex.
- For dust transfer 0.2 mL from the solution into 1.8 mL (10^{-2}) peptone/tween and vortex. Pipette 1.0 mL from the solution into 9.0 mL peptone/tween and vortex. Follow this dilution scheme for 10^{-3}, 10^{-4}, 10^{-5}.
- Pipette 0.2 mL onto each replicate plate and spread with a sterile single use device.
- If multiple plate analysis is requested proceed in the following order: MEA, DG18, SSA, Cellulose.

Swabs

- Swabs are suspended in 2mL (nominal 1:2) of peptone/tween, and placed on a rotary shaker for 2 minutes. Plate 0.2mL onto each replicate plate and spread with sterile spreader. This provides a 10^{-1} dilution set of plates.
- Pipette 1.0 mL from the original mixture into 9.0 mL (10^{-1}) peptone/tween and vortex. Follow this dilution scheme for 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}.
- Plate 0.2mL onto each replicate plate and spread with sterile spreader.

All plates for all analyses are incubated at 25C until an analyst identifies the various fungi growing on the plate (ML009).

EXHIBIT
tabbies 33-B

1337 Capital Circle, Marietta, Georgia 30067 • 770-933-0638 • Fax 678-931-2232 • www.aqs.com
1455 Oliver Road, Suite 240, Fairfield, California 94534 • 707-399-9110 • Fax 707-399-9172

Sampling, Handling and Preservation

- Bulk, swab, and dust samples are collected in the field by an investigator.
- The samples, upon receipt, are labeled with a unique identifier by sample control and delivered to the Microbiology Laboratory.
- Swab samples and wet bulk samples are refrigerated until processing.
- Bulk and dust samples are held in sampling containers submitted at ambient temperatures until processing.
- All processing should be accomplished within 24 hours from the delivery of the samples to the Microbiology Laboratory.
- No preservatives are added to the samples.
- All samples are held a minimum of 1 week before being disposed of by autoclaving and disposed in the normal waste stream.

AIR QUALITY SCIENCES, INC.
Business Solutions for Healthy Indoor Environments

DAVID HAS COME BY FOR A COPY OF THIS REPORT. 354 5460

September 20, 2002

Ms. Sharon Vam
Chatham County Dept. of Public Health
7 Mall Court P.O. Box 14257
Savannah, GA 31416

AQS Report #: 09586-01

Dear Ms. Vam:

Thank you for choosing Air Quality Sciences, Inc. (AQS), an ISO 9002 registered testing laboratory, for your analytical needs. Air Quality Sciences, Inc. is accredited by the American Industrial Hygiene Association (AIHA) in the environmental microbiology program.

Enclosed you will find the analytical results for the samples received on September 5, 2002. Also enclosed is a copy of the field sampling log acknowledging receipt of these samples. Reported data were obtained from samples and sampling information as provided by the on-site investigator. These data and general information are provided to assist the investigator in an overall IAQ assessment. AQS employees did not collect samples nor visit the site where samples were collected. Interpretation of data is left to the client or persons who conducted the field work.

Sources of additional information are also available from:

1. Morey, P. R., Horner, W. E., Epstien, B. L., Worthan, A. G., and Black, M. S. Indoor Air Quality in Nonindustrial Occupational Environments, in R. L. Harris (Ed.) Patty's Industrial Hygiene (5th ed., pp. 3149-3241). John Wiley & Sons: NY, 2000.
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We appreciate the opportunity to assist you. If you have any questions or comments, please feel free to contact our Customer Service Department at (770) 933-0638.

Sincerely,

W. Elliott Horner, Ph.D.
Microbial Laboratory Director

Enclosure

1337 Capital Circle
Atlanta, Georgia 30067
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EXHIBIT
tabbies
33-C

AIR QUALITY SCIENCES, INC.
Business Solutions for Healthy Indoor Environments

MICROBIOLOGICAL LABORATORY RESULTS
CULTURABLE FUNGI FROM BULK SAMPLES (DILUTION)

PREPARED FOR: CHATHAM CO. DEPT. OF PUBLIC HEALTH
AQS PROJECT: 09586

AQS Sample Identification	Customer Sample Identification	Sample Date	Sample Weight (g)	Fungi (Malt Extract Agar) at 25°C	
				CFU/g [a]	Taxa [b, c]
09586-010AAz, BF1m	1	8-02	0.500	>3.25x10^5	Stachybotrys chartarum (>40) unidentified (pigmented) (16) Aspergillus niveus (3) Aspergillus puniceus (3) Aspergillus versicolor (2) Cladosporium sp. (1)
09586-010AAz, BF2m	2	8-02	0.386	>2.59x10^6	Cladosporium sp. (>250) Stachybotrys chartarum (>250) yeast (15) Aureobasidium sp. (1) Alternaria sp. (1)

date sample received: September 5, 2002
date analysis completed: September 20, 2002

[a] Colony forming units per gram of sample.
[b] Taxa listed in descending order of occurrence.
[c] () = Colony count.
[d] Colonies crowded, numerous; accurate count not feasible, actual number likely higher.
[e] Accurate count not possible due to rapid overgrowth; actual number likely higher.
[f] Plate overgrown, taxon present but colonies obscured.
[g] Subculture not successful, ID not possible.
[h] Subculture successful but no acceptable species match in current references.

Limit of detection (LoD) is presented as the calculated concentration for recovery of a single colony for the indicated sample volume and processing conditions. Sample suspension prepared and diluted in laboratory; dilutions replicate plated on indicated culture medium.

Reported data were obtained from samples and sampling information as provided by the on-site investigator. These data and general information are provided to assist the investigator in an overall IAQ assessment. AQS employees did not collect samples nor visit the site where samples were collected. Interpretation of data is left to the client or persons who conducted the field work.

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Fungal Fragments as Indoor Air Biocontaminants

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The aerosolization process of fungal propagules of three species (*Aspergillus versicolor*, *Penicillium melinii*, and *Cladosporium cladosporioides*) was studied by using a newly designed and constructed aerosolization chamber. We discovered that fungal fragments are aerosolized simultaneously with spores from contaminated agar and ceiling tile surfaces. Concentration measurements with an optical particle counter showed that the fragments are released in higher numbers (up to 320 times) than the spores. The release of fungal propagules varied depending on the fungal species, the air velocity above the contaminated surface, and the texture and vibration of the contaminated material. In contrast to spores, the release of fragments from smooth surfaces was not affected by air velocity, indicating a different release mechanism. Correlation analysis showed that the number of released fragments cannot be predicted on the basis of the number of spores. Enzyme-linked immunosorbent assays with monoclonal antibodies produced against *Aspergillus* and *Penicillium* fungal species showed that fragments and spores share common antigens, which not only confirmed the fungal origin of the fragments but also established their potential biological relevance. The considerable immunological reactivity, the high number, and the small particle size of the fungal fragments may contribute to human health effects that have been detected in buildings with mold problems but had no scientific explanation until now. This study suggests that future fungal spore investigations in buildings with mold problems should include the quantitation of fungal fragments.

Water damage in buildings is common and is often associated with mold problems. In North America, cross-sectional questionnaire studies have found that 27 to 36% of homes have mold problems (9, 51). Studies that included indoor air quality measurements have shown even higher numbers, from 42 to 56% (8, 11). In Europe, the prevalence of damp and moldy homes has been reported to be 17 to 46% for Great Britain (5, 23, 34, 42), 15 to 18% for The Netherlands (3, 55, 56), and 15% for Finland (41). Alarmingly, signs of present or previous moisture-related defects were found in 80% of randomly selected private homes investigated by civil engineers trained to recognize the signs of water leaks or condensation (37).

Increased prevalence of water-damaged buildings and subsequent fungal contamination may contribute to the noted increase in allergic diseases. Fungi can affect human health in a variety of ways. Possible reactions generally fall into one of three groups: allergic reactions (sensitization and immune responses, i.e., asthma, allergic rhinitis, or hypersensitivity pneumonitis), infections (growth of the fungus in or on the body, e.g., aspergillosis), and toxic responses (24, 30, 45). The toxic reactions are mainly connected with the secondary fungal metabolites, i.e., mycotoxins, but the role of cell wall components, such as β-(1→3)-D-glucans, has also been reported (4, 26, 29, 45, 47, 50). In addition, exposure to volatile organic compounds produced by fungi while growing on substrates and degrading them may be responsible for nonspecific symptoms, such as headaches; eye, nose, and throat irritation; and fatigue (4, 35).

An association between the existence of mold problems in buildings and adverse health effects has been found in several studies (24, 57). Although epidemiological studies show that people living or working in buildings with mold problems have more respiratory symptoms and diseases than people in nonproblem buildings, the relationship between inhaled fungal spores and induction of respiratory symptoms is still indeterminate and controversial in many aspects (7, 25, 35, 40, 45, 54). While the health problems in moldy buildings are associated with exposure to high concentrations of airborne fungal spores in a few studies (42, 58), several field studies show that the fungal spore concentration in problem buildings is not necessarily higher than that in nonproblem ones (14, 25, 36, 53). This suggests that airborne fungal spores may not be the only agents contributing to the health effects in damp indoor environments.

While some reports are available on the emission of fungal spores from moldy surfaces (16, 18, 39), the release of other types of fungal propagules has not been sufficiently explored. In our recent investigation on the release of fungal spores, performed in the laboratory under controlled environmental conditions (18), we found that parameters such as fungal species, air velocity above the surface, texture of the surface, and vibration of contaminated material affected the release of spores. The principal new finding reported here is that fungal fragments are released together with spores from contaminated surfaces. While the presence of fragments is docu-

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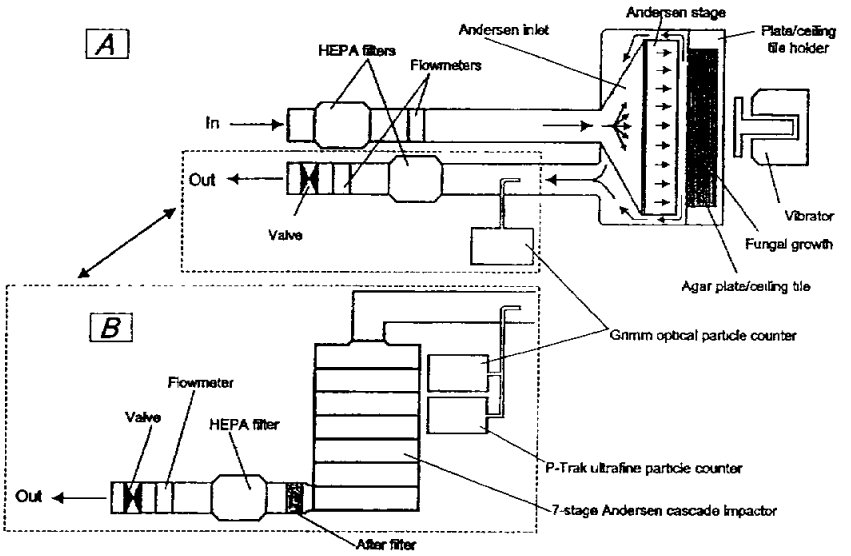

FIG. 1. Experimental setup (A) and its modification for testing the immunological reactivity of fungal propagules (B).

mented with pollen exposures (43, 52), fungal fragments have gained much less attention. The role of fungal fragments is particularly interesting in the light of recent epidemiological studies on the relationship between outdoor air particulate pollution and health effects. These studies present evidence that fine particulates (size, <2.5 μm) are more strongly related to adverse health outcomes than coarse particles (10, 31, 48). The present paper characterizes the release of fungal fragments from contaminated surfaces and compares the results to those obtained in our previous study (18) on the release of fungal spores. The present study also reports data on the immunological reactivity of fungal fragments and spores.

MATERIALS AND METHODS

In the present study the same experimental setup was used and the same test materials and fungal species were selected as in our previous study on the release of fungal spores (18). A brief summary of the procedures is given below.

Experimental setup. The aerosolization chamber and the experimental facility utilized for this study are depicted in Fig. 1 After incubation, the contaminated material (either an agar plate or a ceiling tile) was placed in a holder inside the aerosolization chamber. Fungal fragments and spores from the contaminated material were released by passing clean, HEPA-filtered air over the surface (12144 HEPA capsule filter; Pall Gelman Laboratory, Ann Arbor, Mich.) with controlled airflow rates. The entire setup was placed inside a class II biosafety cabinet (SterilchemGARD, Baker Company, Sanford, Maine). A HEPA filter in the exit flow collected all remaining released propagules to prevent contamination of the room environment.

The experiments were conducted at four air velocities typical for the following environments: indoor air (0 3 m s^{-1}), outdoor air (1.4 and 5 8 m s^{-1}), and ventilation ducts (29.1 m s^{-1}). These four velocities were adjusted through four combinations of two different orifice sizes for the air inlet and two different flow rates through the inlet. Two 400-orifice stages of the 6-stage Andersen impactor (Model 10-800, Andersen Instruments, Atlanta, Ga.) with an orifice diameter of 1 18 and 0.25 mm, respectively, were utilized as the air inlets, one at a time. By virtue of the pressure drop across the 400 nozzles, the airflow through each of the 400 air jets approaching the test surface was the same The airflow rates were 7 and 35 liters min^{-1}.

To investigate the influence of mechanical disturbance on fungal spore release, some tests were performed by applying vibration to the surface at a frequency of 1 Hz at a power level of 14 W. This frequency was selected because it is believed to cause a maximum vibration-induced structural response in buildings (27, 49). A simple electromagnet with an oscillating cylindrical hammer inside was used as the vibrator in the experiments. A sweep/function generator (Model 180; Wavetek, San Diego, Calif.) was connected to the electromagnet to generate the specific combination of frequency and power

The concentration of released propagules was measured with an optical particle counter (Model 1 108; Grimm Technologies, Inc , Douglasville, Ga.). This device, based on light scattering, measures the concentration of particles in the (optical equivalent) size range of 0 3 to 20 μm. The duration of each experiment was 30 min At the beginning of every experiment the system was operated in the absence of any test material in the chamber until the particle level was zero, as measured by the optical particle counter. In the next step, a noncontaminated agar plate or ceiling tile (incubated under the same conditions and times as the inoculated materials) was placed in the aerosolization chamber to establish the background level for particles released from the test surface when exposed to airflow and/or vibration These levels were negligibly low, about 0 01% of the total released propagules. During all the release experiments in a biosafety cabinet, the temperature and relative humidity, measured with a humidity/temperature meter (Fisher Scientific Company, Pittsburgh, Pa.), were 20 to 24°C and 32 to 40%, respectively This low humidity was chosen for the experiments to represent the worst-case scenario, as fungal propagules have been shown to be aerosolized more easily when the air is dry (in contrast to release into humid air) (16, 39). Each test was repeated three to six times Before each test, the experimental system was purged by passing clean air through it.

Tested surface materials. Two surface materials were tested for the release of fungal propagules (i e , fragments and spores) agar plates filled with malt extract agar (Becton Dickinson Microbiology Systems, Sparks, Md) and white ceiling tiles (Armstrong World Industries, Inc., Lancaster, Pa). The latter material is commonly used in buildings in the United States and consists of human-made mineral fibers The porous texture of this material was expected to support fungal growth (15). The tested agar and ceiling tile surfaces had the same round shape and the same dimensions as a plastic petri dish (diameter, 8.7 cm, height, 1 4 cm, area, 59 42 cm^2) Both tested materials were sterilized before being prepared for the experiments. The agar plates were prepared according to the microbiological procedure recommended by the manufacturer (Becton Dickinson Microbiology Systems). Precut pieces of ceiling tiles were autoclaved at 121°C for 15 min. After sterilization, the agar plates and ceiling tiles were inoculated with specific fungal species.

Fungal species and growth conditions. On the basis of earlier investigations (1, 44) and similar to our spore release study (18), three fungal species were selected for the tests *Aspergillus versicolor*, *Penicillium melinii*, and *Cladosporium cladosporioides A versicolor* and *C. cladosporioides* are commonly present in indoor air (6, 17, 19, 28, 32, 38, 40) *P. melinii* is characteristic of soil environments. This species has, however, previously been isolated from contaminated building materials and was selected to represent fungi with large spores (44).

The fungal species were first grown on malt extract agar plates at 24°C at a relative humidity of 32 to 40% for 7 days before inoculation of the test materials

Fungal suspensions were prepared by washing fungal colonies from the agar plates with deionized and sterilized water (5 Stage Milli-Q Plus System, Millipore Corporation, Bedford, Mass.). The spore concentrations in the initial water suspensions were checked by using a bright line hemacytometer counting chamber (Model 3900; Hausser Scientific Company, Horsham, Pa.), and the concentration was adjusted to 10^6 spores per ml. The agar plates and ceiling tiles were inoculated with 0.1 and 1 ml of fungal suspensions, respectively. After inoculation, the agar plates and ceiling tiles were incubated in separate chambers at 24°C and a relative humidity of 97 to 99%. This humidity was achieved by placing saturated K_2SO_4 solution (150 g per liter) at the bottom of the incubation chambers (20). The agar plates were incubated for 7 days and the ceiling tiles were incubated for 6 (*C. cladosporioides* and *P. melinii*) or 12 (*A. versicolor*) months, which resulted in abundant fungal growth on both surfaces. Temperature and humidity in the chambers were monitored by a humidity/temperature meter.

After incubation, two samples of each of the two tested materials were used to determine the initial spore surface concentration. A 2-cm^2 piece of the contaminated material was cut and suspended in 25 ml of deionized and sterilized water in a test tube. The spores were then extracted from the material by vortexing them for 10 min in a vortex touch mixer (Model 231, Fisher Scientific Company, Pittsburgh, Pa.). The spore concentrations in the resulting suspensions were determined with the bright line hemacytometer, which indicated about 10^7 spores per cm^2 for both agar and ceiling tile samples. The hyphal structure of the fungal colonies on the agar and ceiling tile surfaces was observed by using both a light microscope (Model Labophot 2A, Nikon, Tokyo, Japan, available through Fryers Company, Inc., Carpentersville, Ill.) and a stereomicroscope (Model Stereomaster II; Fisher Scientific Company).

SEM analysis. The presence of fragments was confirmed by scanning electron microscope (SEM) analysis. For this purpose, fungal propagules were aerosolized and sampled during 30-min experiments onto a 25-mm polycarbonate membrane filter with a pore size of 0.2 μm (Millipore Co.) with an in-line filter holder (Pall Gelman Laboratory), which replaced the HEPA filter in the outlet tube downstream of the aerosolization chamber (Fig. 1A). After sampling, the polycarbonate filters were coated with platinum (JEOL JFC-1300 auto fine coater metalliser; JEOL, Tokyo, Japan) and then analyzed by using low-vacuum SEM (Model JSM 5600LV; JEOL) paired with Oxford microanalysis. The secondary vacuum in the SEM operated at a pressure of 10^{-4} Pa. The images obtained during the analysis were digitized from an electron detector of the SEM and were passed to the computer.

Immunological reactivity of fungal propagules. To test the immunological reactivity of *A. versicolor* and *P. melinii* propagules, the fragments and spores were simultaneously released from the agar plates at an air velocity of 24 m s^{-1} and were then collected onto separate filters by using a cascade impactor. For the purpose of these experiments, a 7-stage Andersen Cascade Impactor (Andersen Instruments Inc., Smyrna, Ga.) was added to the experimental setup (Fig. 1B). This device has particle cutoff sizes of 7.4, 4.7, 3.3, 2.1, 1.1, 0.65, and 0.43 μm for stages 1 through 7, respectively. The impaction plates of stages 3 to 7 were covered with a double-sided sticky tape (Manco Inc., Westlake, Ohio), which was discarded after each experiment. This was done to decrease particle bounce and to improve the separation of fragments and spores. Impaction stages 1 and 2 (which collected most of the spores) had 80-mm-diameter polyvinyl chloride (PVC) filters as substrates (Omega Specialty Instrument Co., Chelmsford, Mass.). The remaining propagules, most of which were fragments, were collected onto a 37-mm-diameter PVC filter with a pore size of 0.8 μm (SKC Inc., Eighty Four, Pa.). This filter was placed directly after the impactor outlet and is marked "After filter" in Fig. 1B.

Each set of samples was collected for 4 h. During this time the fungal propagules were released from 24 contaminated agar plates, which were changed every 10 min in order to attain sufficiently high concentration of released fungal propagules. The latter was measured simultaneously by using the Grimm optical particle counter and an ultrafine particle counter (P-Trak, Model 8525; TSI Incorporated, St. Paul, Minn.). The P-Trak is a condensation nuclei counter, which measures the concentration of particles in the size range of 0.02 μm to greater than 1 μm.

For the immunological reactivity tests, only the filter from stage 2 (collecting spores and their agglomerates in the 4.7- to 7.4-μm size range) and the after filter (collecting fragments) were used. After collection, each PVC filter was cut up, placed separately in a safe-lock Eppendorf micro test tube (Brinkmann Instruments, Inc., Westbury, N.Y.), and soaked with 1 ml of carbonate coating buffer at a pH of 9.6. The collected fungal propagules were suspended from the filters by vortexing for 0.5 min with a vortex mixer (Model Vortex-Genie 2; Scientific Industries, Bohemia, N.Y.). To guarantee purity (absence of spores) in the fragment samples, the fragment suspensions were filtered through 25-mm-diameter polycarbonate membrane filters with a pore size of 2 μm (Millipore Co.). The purity of the fragment suspensions as well as the spore concentrations present in the spore suspensions were checked by using a bright line hemacytometer counting chamber. The number of released fragments with diameters below 0.4 μm (collected on the after filter) was estimated by subtracting the number of particles within the 0.4- to 1-μm size range (recorded by the Grimm optical particle counter) from the number of particles within the 0.02- to 1-μm size range (recorded by the P-Trak ultrafine particle counter).

The immunological reactivity of the fungal propagules was tested by using a modified enzyme-linked immunosorbent assay (ELISA). The fungal fragment and spore suspensions (100 μl) were pipetted into the wells of ELISA MicroWell plates (Nalge Nunc International, Naperville, Ill.) and were incubated overnight at room temperature. After incubation the wells were washed twice with 200 μl of phosphate-buffered saline containing 0.05% Tween 20 (PBST). The ELISA plates were then processed according to the following five sequential steps, each step being separated from the next by two washing steps with PBST: (i) incubation for 1 h at room temperature in 200 μl of PBST containing 1% nonfat milk powder (PBSTM), (ii) incubation for 1 h at 37°C in 100 μl of monoclonal antibody (MAb) culture supernatant diluted five times into PBSTM; (iii) incubation for 1 h at 37°C in 100 μl of Biotin-SP-conjugated AffiPure goat anti-mouse immunoglobulin G plus immunoglobulin M secondary antibody (Jackson ImmunoResearch Laboratories, Inc., West Grove, Pa.) at a dilution of 1/5,000 in PBSTM; (iv) incubation for 1 h at 37°C in 100 μl of alkaline phosphatase-conjugated streptavidin (Jackson ImmunoResearch Laboratories, Inc.) at a dilution of 1/5,000 in PBSTM; and (v) incubation for 30 min at room temperature in 100 μl of substrate buffer (97 ml of diethanolamine, 100 mg of $MgCl_2$ [both from Sigma Chemical Co., St. Louis, Mo.] in 1 liter of distilled water; pH was adjusted to 9.8 with HCl) containing one 5-mg *p*-nitrophenyl phosphate tablet (Sigma Chemical Co.) in 10 ml of buffer. Three different MAbs were used in the tests: MAb 14F7 produced against *A. versicolor* but cross-reacted with *P. melinii*, MAb 5F7 produced against *Penicillium brevicompactum* but cross-reacted with *A. versicolor* and *P. melinii*, and MAb 12G2 produced against *Penicillium chrysogenum* but cross-reacted with *A. versicolor* and *P. melinii*. After incubation, the absorbance of the prepared samples was read spectrophotometrically at a wavelength of 405 nm (UltraMicroplate Reader, Model EL_x800; BIO-TEK Instruments, Inc., Winooski, Vt.). Two independent sets of samples for each tested fungal species of *A. versicolor* and *P. melinii* were tested in triplicate (one set is a pair of one spore and one fragment sample). Two blank PVC filters used as negative controls were tested in parallel with the sample filters.

Data analysis. The data were statistically analyzed by analysis of variance and *t* test by using the software package STATISTICA for Windows (StatSoft, Inc., Tulsa, Okla.).

RESULTS

The optical particle size distribution was measured for each tested fungal species at an air velocity of 29.1 m s^{-1} with both agar and ceiling tile samples. The results are presented in Fig. 2. Previous reports indicate that the physical size of spores (measured under a microscope) is 2 to 3.5 μm for *A. versicolor* (close to spheres), 3 to 2 μm by 7 to 4 μm for *C. cladosporioides* (ellipsoidal shape), and 5 to 6 μm for *P. melinii* (close to spheres). The respective aerodynamic equivalent sizes are 2.5, 1.8, and 3.0 μm (44). The optical size distributions of the released fungal propagules, shown in Fig. 2, were the first ones to reveal that particles significantly smaller than the single spore size are released from the cultures of all three tested fungal species. This is clearly seen as an additional peak in the submicrometer size range. The peak in the number of released particles corresponding to the spore size for all test organisms is seen between 1.6 and 3.0 μm in equivalent optical diameter. Therefore, the particle size of 1.6 μm was selected as the lower counting limit separating spores from fungal fragments.

The presence of fungal fragments was confirmed by collecting filter samples and investigating them under the SEM. Figure 3 displays an example of fragments and spores released from *A. versicolor* culture. Simultaneous release of intact

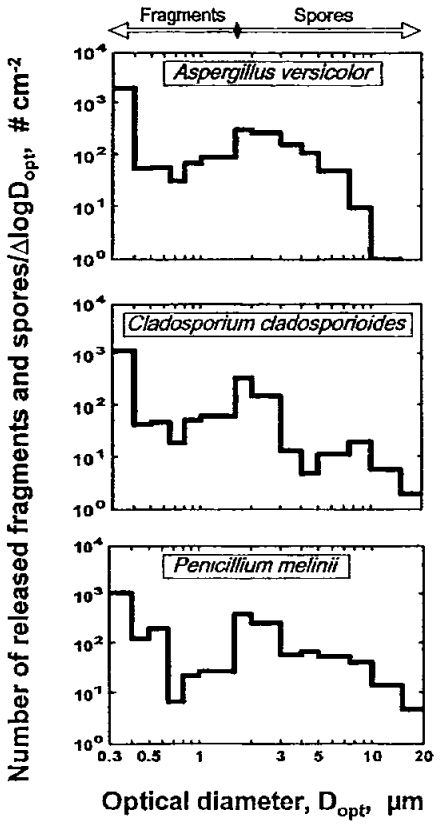

FIG. 2. Optical size distribution of *A. versicolor*, *C. cladosporioides*, and *P. melinii* propagules released from both agar and ceiling tile surfaces (composite values) during 30-min experiments.

spores (Fig. 3A) as well as fragmented spores and/or hyphal fragments (Fig. 3B) was observed.

Figure 4 shows a comparison between the number of fragments and spores released from agar surfaces at four air velocities. The number of released fragments ranged from 160 to 1,400 particles per cm^2, and the number of spores ranged from 1 to 70 per cm^2. Although the optical particle counter used in this study detects and counts particles only down to 0.3 μm, the data indicate that concentrations of released fragments were 11 to 320 times higher than those for spores of *A. versicolor*, 17 to 170 times higher than those for spores of *C. cladosporioides*, and 7 to 270 times higher than those for spores of *P. melinii*. All these differences were statistically significant ($P < 0.05$ for *A. versicolor* and *C. cladosporioides* and $P < 0.000001$ for *P. melinii* by *t* test). The lowest fragment/spore ratio was usually observed for an air velocity (v) of 29.1 m s^{-1}, and the highest ratio varied depending on the fungal species (v = 5.8 m s^{-1} for *A. versicolor*, v = 1.4 m s^{-1} for *C. cladosporioides*, and v = 0 3 m s^{-1} for *P. melinii*).

Our recent findings (18) reveal that the spore release from agar increased with increasing air velocity. In this study, air velocity did not affect the number of released fragments ($P > 0.05$ by analysis of variance), and therefore further experiments were performed at the two extreme velocities, 0.3 m s^{-1} (typical air velocity in indoor environments) and 29.1 m s^{-1} (typical air velocity in ventilation ducts).

Table 1 compares the results on the release of fragments and spores from agar and ceiling tile surfaces at the two air velocities indicated above. Similar to what was determined about the release from agar, the number of aerosolized fungal fragments from ceiling tile surfaces was always higher than the number of released intact spores. At an air velocity of 29.1 m s^{-1} the release of fragments from ceiling tiles was much higher than that from agar surfaces, reaching its maximum of 5.7 × 10^5 particles per cm^2 for *P. melinii* ($P < 0.000001$). At a lower air velocity of 0.3 m s^{-1} the release rate reached 2.4 × 10^3 fragments per cm^2, but no significant differences were observed for the number of fragments released from these two surfaces. In contrast to agar, there was a noticeable increase in the number of released fragments for all tested fungal species with increased air velocity from the ceiling tile surfaces. The *t* test statistically confirmed these differences for fragments of *A. versicolor* ($P < 0.05$) and *P. melinii* ($P < 0.001$). Similar release trends were noted for fungal spores (18).

Data on the effect of surface vibration on the release of fungal fragments are also shown in Table 1. Vibration of ceiling tiles at the lower air velocity of 0.3 m s^{-1} increased the release of *Cladosporium* ($P < 0.05$) and *Penicillium* fragments ($P < 0.01$). For *Aspergillus* fragments this difference was not statistically significant. At v = 29.1 m s^{-1} no statistically significant effect of vibration on the release of fragments was observed. The same observation was previously concluded for the spore release experiments (18). Similar to the tests conducted without vibration, the augmentation of air velocity from 0.3 to 29.1 m s^{-1} resulted in an increase in the number of released fungal fragments from ceiling tiles when vibration was applied. Statistical analysis (*t* test) confirmed this trend for fragments of *A. versicolor* ($P < 0.01$) and *P. melinii* ($P < 0.0001$).

The percentage of released fungal fragments and spores during the first 10 min of the 30-min experiments is presented in Table 2. These data were obtained at airflow velocities of 0.3 and 29.1 m s^{-1} for agar and ceiling tiles without vibration applied and for ceiling tiles when these two air flows were accompanied by vibration. For all these species, the percentage of released fragments was 30 to 53% and of released spores was 27 to 45% at the air velocity of 0.3 m s^{-1} when no vibration was applied. When vibration was applied to the ceiling tile surfaces the respective mean percentage increased to 51 to 53% for the release of fragments and to 59 to 76% for the release of spores. At the air velocity of 29.1 m s^{-1} the mean percentage of released fungal propagules increased to 66 to 86% for fragments and to 71 to 88% for intact spores. Applying the surface vibration at this air velocity did not affect the fragment and spore release (the same mean values of 76 and 81%, respectively).

The correlation between the numbers of released fungal propagules was analyzed for the three tested fungal species. For each species, all the data on the number of released fungal fragments (from agar and ceiling tiles with and without vibration) at an air velocity of 0.3 m s^{-1} were grouped into one category and were correlated with the respective numbers of released spores. The data obtained at an air velocity of 29.1 m s^{-1} were grouped into another category and were tested separately. The results are summarized in Table 3. As shown, strong correlations were observed at the air velocity of 29.1 m

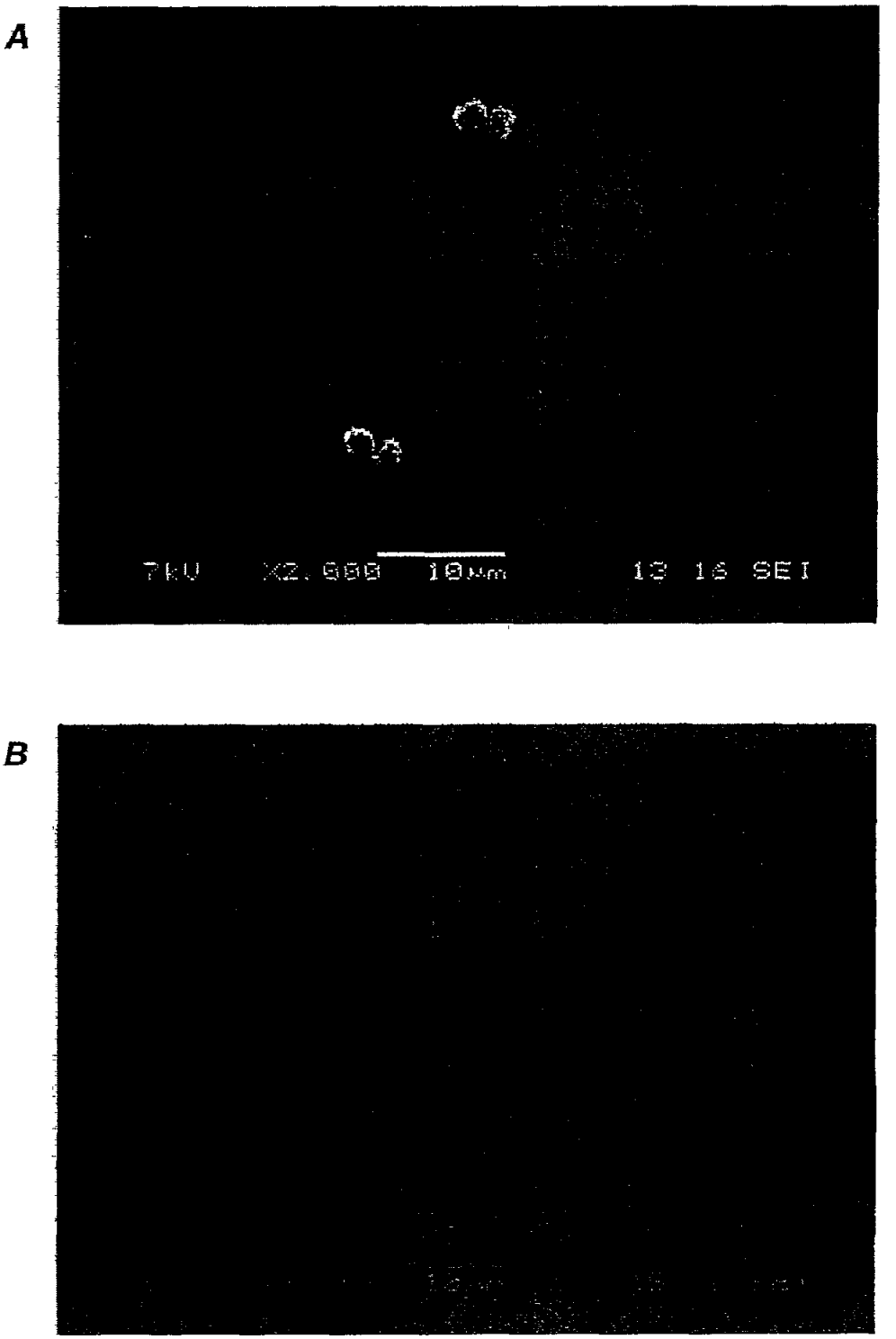

FIG. 3. SEM pictures of propagules released from a ceiling tile contaminated with *A. versicolor* (panel A, intact spores; panel B, fragments).

s^{-1}; for all three species the correlation coefficients were close to 1 ($P < 0.05$). At the air velocity of 0.3 m s^{-1}, the correlation between fragments and spores was found to be statistically significant ($r^2 = 0.508$, $P < 0.05$) only for *P. melinii*.

The immunological reactivities of *A. versicolor* and *P. melinii* fragments and spores are shown in Fig. 5. The total number of fragments versus spores in *A. versicolor* samples were as follows: sample 1, 4.7×10^6 versus 1.4×10^4, sample 2, 1.7×10^7

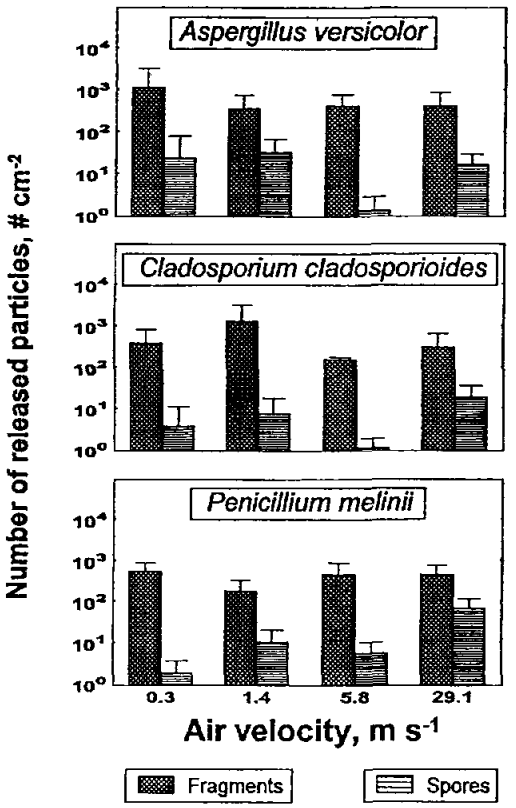

FIG. 4. Number of fungal fragments and spores released simultaneously from agar surfaces at four different air velocities during 30-min experiments. The error bars indicate the standard deviation of 6 repeats for *A. versicolor*, 4 repeats for *C. cladosporioides*, and 10 repeats for *P. melinii*. The spore data are taken from Górny et al. (18).

versus 1.3×10^4. The respective numbers for *P. melinii* were as follows: sample 1, 1.8×10^8 versus 1.6×10^5; sample 2, 1.0×10^7 versus 1.0×10^5. The immunological reactivity was expressed as the optical density of the respective fungal propagule sample incubated with a MAb (λ = 405 nm after 30 min of substrate incubation time). The fragment and spore samples always showed significant immunological reactivity independent of the type of MAb used. For the tested *A. versicolor* fragment samples the optical densities were 3.7 to 5.1 times higher than those for the spore samples (*P* value in *t* test varied from <0.05 to <0.01). For the *P. melinii* fragment samples the respective values were 2.0 to 3.2 times higher than those for the spore samples (always with a *P* value of <0.01). The blank filter samples (negative controls) tested simultaneously with the fragment and spore samples showed no activity in ELISA.

DISCUSSION

The most interesting finding of this study was that a significant amount of immunologically reactive particles having sizes considerably smaller than those of the spores was released from surfaces contaminated with fungi. Even with the accuracy of the Grimm optical size spectrometer, which allows measurement of particles as small as 0 3 μm in size, these fragments outnumbered the aerosolized spores by up to 320 times. The presence of fragments was confirmed by SEM observations.

The presence of airborne fragments is clearly documented with pollen exposures, as the onset of seasonal allergies is shown to start several weeks before the respective pollen grains are detected in the air (43, 52). In contrast, the role of fragments in fungal exposures has not been sufficiently recognized. The reason for this may be that fine and ultrafine fragment particles cannot be detected with traditional bioaerosol sampling and analysis methods. However, previous reports on mycelium pieces (that were large enough to be detected by light microscopy) indicate the possibility of the presence of fragments. Li and Kendrick (32) and Robertson (46) showed that the concentrations of fungal fragments in indoor air can reach an average level of 29 to 146 pieces per m^3, i.e., up to 6.3% of all fungal propagules indoors. Madelin and Madelin (33) reported that pieces of mycelium are often blown away from contaminated surfaces, and some of these pieces remain viable and capable of initiating new growth. It is also possible that the fragments are pieces of spores and fruiting bodies or are formed through nucleation from secondary metabolites of fungi, such as semivolatile organic compounds.

Some researchers have compared the allergic responses of spore and mycelial extracts and have found that they share common allergens but that their reactivities vary and, in some cases of extract comparison, the intensities of the reactions from mycelium can exceed those obtained from the comparable spore extract studies (2, 12, 13). Our findings seem to be in good agreement with these results. All three tested MAbs revealed reactivity with the examined fungal propagules. The finding that the fragment samples had fungal antigens even after filtering for the remaining spores confirms that the fragments were indeed of fungal origin. Furthermore, the activity of the fungal fragment samples always exceeded that obtained for the spore samples. The reported numbers of fungal fragments and spores were released from the same area of contaminated surfaces during the same sampling time and thus represent a true exposure situation. The high number and reactivity of the fungal fragments are striking, suggesting that these fragments may significantly influence the health of exposed individuals. This factor has, to the best of our knowledge, been overlooked so far in studies evaluating indoor air quality. The specificity and the cross-reactivity of the tested MAbs should be taken into consideration when evaluating the above results. In our studies, the activity of *P. melinii* fragments and spores was higher than that of *A. versicolor* propagules. The high optical density values were probably caused by the higher number of antibody-specific fungal antigens present in the tested propagule suspensions. However, the high reactivity of fungal fragments itself appears to be of great significance from an exposure assessment point of view. As seen from the data given above, the high number of small particles being immunologically reactive and penetrating into the human respiratory tract can potentially be the cause of adverse health effects and can, at least in part, be responsible for unexplained cases of respiratory symptoms in damp buildings.

The results of previous studies (18, 21, 39, 59) indicate that the release of fungal spores generally increased when the air velocity above the contaminated surface increased. In the present study, however, the release of fragments from smooth

TABLE 1. Average number of fungal fragments and spores released simultaneously from agar plates and ceiling tiles during 30-min experiments

Species	Type of surface	No. of fragments and spores (cm^{-2}) released at the following air velocity[a]							
		0.3 m s^{-1}				29.1 m s^{-1}			
		Fragment		Spore[b]		Fragment		Spore[b]	
		Avg	SD	Avg	SD	Avg	SD	Avg	SD
A. versicolor	Agar plate without vibration	1,240[c]	2,270[c]	26[c]	60[c]	441[c]	491[c]	18[c]	14[c]
	Ceiling tile without vibration	2,390[c]	229[c]	101[c]	69[c]	129,000[c]	103,000[c]	44,800[c]	40,600[c]
	Ceiling tile with vibration	1,150	875	361	315	144,000	52,000	46,400	17,100
C. cladosporioides	Agar plate without vibration	426	473	4	8	331	375	20	19
	Ceiling tile without vibration	85	41	2	2	19,300	16,600	4,590	3,950
	Ceiling tile with vibration	429	175	70	66	129,000	99,100	42,400	34,300
P. melinii	Agar plate without vibration	604[c]	355[c]	2[c]	2[c]	487[c]	343[c]	74[c]	52[c]
	Ceiling tile without vibration	36[c]	23[c]	5[c]	3[c]	571,000[c]	146,000[c]	420,000[c]	170,000[c]
	Ceiling tile with vibration	1,550	690	463	98	646,000	53,200	509,000	53,700

[a] The numbers represent the average value and standard deviation of three repeats, unless indicated otherwise
[b] Fungal spore release data are from Górny et al. (18)
[c] Average value for four repeats.

agar surfaces was not affected by the air velocity. The different trend in fragment release compared to that of intact spores indicates that the fragments are aerosolized through a process different from that for spores. It is hypothesized that the fragments are already liberated from the mycelium or spores before the air currents carry them away. Thus, all the fragments are aerosolized at low air velocity, and an increase in the velocity does not increase their release. The increased release from rough ceiling tile surfaces appears to be related to the higher air turbulence effect above the surface cavities (18). The particular components of fungi (hyphae, conidiophores, and spore chains) overgrew almost the entire surface on both materials. Stereomicroscopic observations revealed that for ceiling tiles, growth occurs not only on the top surface, but the fungal colonies grow in each of the surface cavities as well. Fungal mycelium rises vertically upward, creating a mesh-like structure in the recesses of the ceiling tile surface. The higher air velocity with increased turbulence is more likely than the lower air velocity to release fungal propagules from the surface cavities. The difference in the fragment release between agar and ceiling tile can be partially caused by the differences in the moisture conditions. Moisture from agar can penetrate the thick layer of fungal growth and thus can increase the adhesion forces and reduce the release of fungal propagules. It should also be noted that the adhesion forces are higher for fungal fragments than for fungal spores due to the smaller size of the fragments.

On the basis of microscopic observations it can also be concluded that the morphology of fungal colonies may play an important role in the fragment release mechanism. *A. versicolor* and *P. melinii* colonies have longer, thinner conidiophores and longer spore chains than the colonies of *C. cladosporioides*. During exposure to the air currents, elongated *Aspergillus* and *Penicillium* colony parts (conidiophores, metulas, and phialides) as well as other structural elements (e.g., joint areas between the spores) are much more susceptible to desiccation stress (because of the larger exposed area) than the respective *Cladosporium* structures, and they probably become much more brittle when subjected to air turbulence.

In the real world ceiling tiles are probably under constant influence from different sources of vibration. This mechanical disturbance can originate both from the indoors and outdoors (operating home appliances, heating or air conditioning units, residents' activities, wall vibrations caused by road traffic, ground movement, etc.) and generally may lead to particle release from surfaces into the air (22). The vibration parameters selected for this study, i.e., a frequency of 1 Hz at a power level of 14 W, reflect the disturbances caused by, e.g., closing or slamming a door or children jumping. Similar to results obtained for spores (18), the vibration was shown to increase the release of fungal fragments at the low air velocity. This indicates that vibration is an important mechanism affecting the release of fungal propagules in indoor air environments and may partly explain the sporadic release of fungal propagules discussed below. High-velocity air currents appeared to have released all the fragments that were capable of being aerosolized, and therefore additional forces applied by vibration did not increase the release.

Regarding the colony morphology, mechanical stress caused by vibration may also cause additional release of fungal fragments from the elongated colony structures. Shorter structures of *C. cladosporioides* are more compact and, thus, are probably more resistant to the forces created by vibration of the surface. Elongated parts of *A. versicolor* and *P. melinii* colonies appear to be more easily affected by this mechanical force, which results in higher aerosolization of fragments, especially when the air current is low (indoor conditions).

The air currents were able to release up to 86% of the fungal fragments at v = 29.1 m s^{-1} and up to 53% of the fungal fragments at v = 0.3 m s^{-1} from contaminated surfaces during the first 10 min. This is almost the same percentage as that determined earlier for intact spore release (18). On the basis of these results it can be concluded that a significant portion of fungal propagules can be aerosolized from contaminated sur-

TABLE 2. Percentage of released fungal fragments and spores during the first 10 min of 30-min experiments[a]

Species	Type of surface	% of released fragments and spores at the following air velocity:			
		0.3 m s^{-1}		29.1 m s^{-1}	
		Fragment	Spore[b]	Fragment	Spore[b]
A. versicolor	Agar plate without vibration	32	29	84	87
	Ceiling tile without vibration	35	45	86	88
	Ceiling tile with vibration	51	59	77	79
C. cladosporioides	Agar plate without vibration	30	27	66	71
	Ceiling tile without vibration	53	30	79	80
	Ceiling tile with vibration	53	76	81	82
P. melinii	Agar plate without vibration	37	32	66	76
	Ceiling tile without vibration	41	30	77	86
	Ceiling tile with vibration	53	72	71	83

[a] After 30 min, spore and fragment release were 100%
[b] Fungal spore release data are from Górny et al (18)

faces during a very short time interval. Such a high concentration of particles generated during a relatively short time period can significantly contribute to the indoor air quality. It is well known that fungal spore concentrations in indoor air have a wide temporal variation. Our study indicates that a similar variation is likely to occur with airborne fungal fragments in contaminated buildings.

This study showed that the number of released fragments was always higher than the number of intact spores released from contaminated surfaces. This trend seemed to be the same regardless of air velocity, surface material, and the presence or absence of vibration applied to the surface. On the basis of the correlation analysis of the above-described results it can be concluded that the number of fragments released at high air velocities can be predicted if the number of spores present in the air is known. At low air velocities such predictions could be burdened with significant error.

Conclusions. This study revealed that fungal fragments are aerosolized simultaneously with spores from contaminated surfaces. The released fungal fragments consistently outnumber the spores and can exceed 6×10^5 particles per cm^2. Such changes in the number of potentially immunologically relevant particles should be taken into consideration when performing exposure assessments in indoor environments.

The tests performed with MAbs produced against *Aspergillus* and *Penicillium* fungal species revealed that the fungal fragment and spore suspensions both had immunological reactivity. ELISA tests showed that fragments and spores share common antigens which not only confirmed the fungal origin of the fragments but also established their potential biological relevance. The fragment fraction of released fungal propagules, not previously measured in water-damaged buildings, may contribute to adverse health effects that have been detected among building inhabitants.

While the spore release from surfaces increased with increased air velocity, the release of fragments from smooth agar surfaces was not affected by the magnitude of the air velocity, indicating different release mechanisms for spores and fragments, respectively. At the low air velocity (typical for indoor environments), the application of vibration to the contami-

TABLE 3. Correlation between the numbers of released spores and fragments

Species	Correlation at the following air velocity:			
	0.3 m s^{-1}		29.1 m s^{-1}	
	r^2	P	r^2	P
A. versicolor	0.084	>0.05	0.986	<0.05
C. cladosporioides	0.104	>0.05	0.996	<0.05
P. melinii	0.508	<0.05	0.956	<0.05

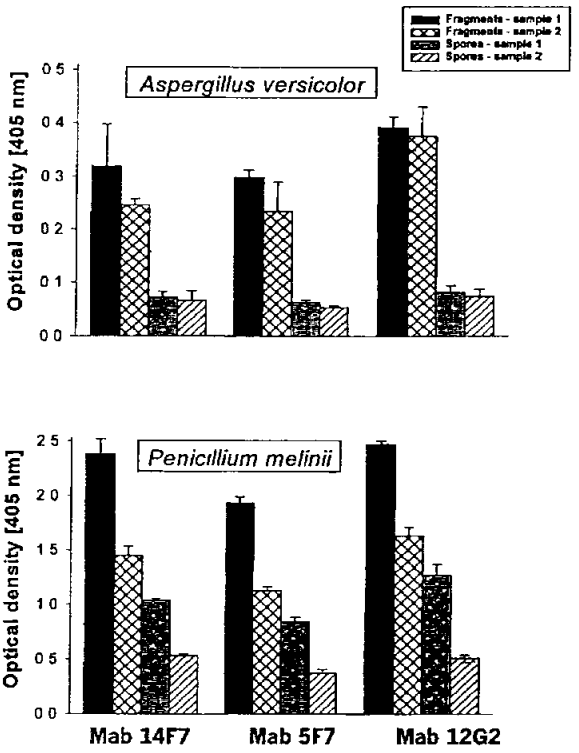

FIG. 5. ELISA reactivity (defined as optical density) of fungal fragments and spores with three MAbs: MAb 14F7, MAb 5F7, and MAb 12G2. The error bars indicate the standard deviation of three repeats.

nated surface increased the number of released particles. Furthermore, our results show that up to 86% of aerosolizable fungal fragments can be rendered airborne during the first 10 min of exposure to airflow. Thus, this study indicates that the concentration of airborne fungal fragments is likely to vary widely, similar to the wide variations in spore concentration in contaminated buildings.

On the basis of the correlation analysis results, we conclude that in indoor environments the number of released spores is generally not a reliable indicator for the number of released fragments. Thus, future studies on mold problem buildings should include the measurement of fungal fragments in addition to intact spores.

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EXHIBIT
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Building-related respiratory symptoms can be predicted with semi-quantitative indices of exposure to dampness and mold

Abstract Using a semi-quantitative mold exposure index, the National Institute for Occupational Safety and Health (NIOSH) investigated 13 college buildings to examine whether building-related respiratory symptoms among employees are associated with environmental exposure to mold and dampness in buildings. We collected data on upper and lower respiratory symptoms and their building-relatedness, and time spent in specific rooms with a self-administered questionnaires. Trained NIOSH industrial hygienists classified rooms for water stains, visible mold, mold odor, and moisture using semi-quantitative scales and then estimated individual exposure indices weighted by the time spent in specific rooms. The semi-quantitative exposure indices significantly predicted building-related respiratory symptoms, including wheeze [odds ratio (OR) = 2.3; 95% confidence interval (CI) = 1.1–4.5], chest tightness (OR = 2.2; 95% CI = 1.1–4.6), shortness of breath (OR = 2.7; 95% CI = 1.2–6.1), nasal (OR = 2.5; 95% CI = 1.3–4.7) and sinus (OR = 2.2; 95% CI = 1.2–4.1) symptoms, with exposure–response relationships. We found that conditions suggestive of indoor mold exposure at work were associated with building-related respiratory symptoms. Our findings suggest that observational semi-quantitative indices of exposure to dampness and mold can support action to prevent building-related respiratory diseases.

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Key words: Dampness; Mold; Semi-quantitative exposure index; Respiratory symptoms; Building; Indoor air quality.

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Introduction

Current airborne fungal sampling and analytical methods provide continuous scales of measurement for examining exposure–response relationships. They are, however, limited by potentially large sampling and analytical error, in addition to spatial and temporal variance of fungal levels (Hyvärinen et al., 2001). Large variability of bioaerosol measurements is likely to produce exposure misclassification in small sample sizes, which may lead to the false conclusion that no association exists between exposure and health effects in epidemiologic studies (Park et al., 2000; Rappaport et al., 1995).

Various studies have linked respiratory symptoms and diseases with damp residential environments and mold growth (Hyndman, 1990; Jaakkola et al., 1993; Mohamed et al., 1995; Nafstad et al., 1998; Platt et al., 1989; Verhoeff et al., 1995). In these analyses, entire buildings or rooms within buildings were frequently

dichotomized into damp/not damp based on one or more of the following factors – visible mold, water stains, condensation, water damage, and mold odor – using self-reports, inspections, or humidity measurements (Bornehag et al., 2001). Such dichotomous distinctions can provide insight about possible associations with health effects, but may not provide adequate information to assess possible exposure–response relationships.

Faced with difficulties in bioaerosol measurement and inadequacies of dichotomous assessment of dampness, semi-quantitative methods of assessing exposure to dampness/mold based on visual and olfactory classification offer a potentially powerful and cost-efficient alternative approach for epidemiologic exposure–response studies of damp building environments (Haverinen et al., 2003).

The National Institute for Occupational Safety and Health conducted an investigation at a college where employees' concerns included building-related respiratory symptoms, asthma, and interstitial lung disease (Schleiff et al., 2002). In this study, we evaluated the presence of building-related respiratory symptoms and diagnoses. We also examined the utility of semi-quantitative dampness/mold exposure indices using observational measurements in predicting respiratory symptoms.

Methods

Study population and epidemiological survey

The college encompassed 40 buildings in which approximately 1231 full-time employees worked. Based on discussions with officials at the college, we selected all seven buildings that had a recurrent history of water incursion and related renovations (water-damaged buildings). We also selected six comparison buildings that were reported to have had little problem with water incursion (comparison buildings). We conducted a self-administered questionnaire survey of 554 full-time employees in these 13 buildings to obtain demographic characteristics, respiratory diagnoses and symptom data, and the time fraction spent in specific rooms. The respiratory question modules were from the European Community Respiratory Health Survey and the American Thoracic Society Questionnaires (EC Directorate General XIII, 1994; Ferris, 1978). The response rate of the full-time employees in the 13 buildings was 71% ($n = 393$). The response rate by primary building type (primary building is defined as the building in which a respondent spent most time during the school year) was 76% in water-damaged buildings and 59% in comparison buildings. We conducted a separate telephone survey of 161 non-participants about 4 months later, and the response rate was 39% ($n = 63$). Among the 393 participants in the main survey, data for 323 employees for whom we had complete information about the amount of time worked in specific rooms during the fall semester were used for statistical modeling. We analyzed lower respiratory (wheeze, chest tightness, shortness of breath, and attacks of cough) and upper respiratory (nasal symptoms, sinus symptoms, and throat irritation) building-related symptoms reported to have occurred in the previous 12 months. Building-related symptoms were defined as those that improved away from work or for which medication use increased on workdays. Our investigation included a 'natural experiment' in which the majority of faculty members from one department had relocated within 8 months before the survey from one of the water-damaged buildings to three of the comparison buildings because of a high prevalence of respiratory illness.

Environmental evaluation

We inspected all 669 accessible rooms on all floors of 12 buildings and a randomly selected 25% ($n = 52$) of the rooms on each floor of the newest comparison building because of time and resource limitation. Using a standardized evaluation form, teams of industrial hygienists classified four environmental factors (water stains, visible mold, mold odor, and moisture) in seven areas of each room. The seven areas were: ceiling; walls; windows; floor; heating, ventilation, and air conditioning system; water pipes; and furniture. To check the validity of observations, two different teams independently cross-classified eight rooms in water-damaged buildings; concordance rates for individual environmental factors were 88% for water stain (from the comparison of dichotomous variables using median average-water-stain score), 63% for visible mold, 75% for mold odor, and 100% for moisture.

We graded water stains on a scale of 0–3 (0 = no water stains; 1 = water stains < 5% of the evaluated area; 2 = 5–30%; 3 = > 30%) for each area. We then averaged the scores over the seven areas within a room to give the average water-stain score (AWSS). Visible mold was documented when seen in any area. Mold odor was graded on a scale from no odor to slight to strong odor. We graded moisture as 'damp' if an area was moist to the touch or as 'wet' if visible water was observed. We also created two combined scores for each room using the environmental factors with different weights: (i) water-stain-weighted combination = 1.0 (if water stains) + 0.5 (if visible mold) + 0.5 (if mold odor) + 0.5 (if damp or wet); and (ii) visible-mold-weighted combination = 0.5 (if water stains) + 1.0 (if visible mold) + 0.5 (if mold odor) + 0.5 (if damp or wet). We gave more weight to either water stains or visible mold in these two combinations as those two environmental factors were most prevalent among all environmental factors.

:timation of individual exposure index and data analysis

/e estimated individual employees' exposure to each ıvironmental factor or combinations of the environ-ıental factors by the following equation:

$$\text{Individual exposure index (IEI)} = \sum_{i=1}^{k} (E_i \times \text{TF}_i),$$

here i = specific room; $E_i = 0$ (none) vs. 1 (any) for ıdividual environmental factors, or continuous value or AWSS or each combination of four environmental ıctors, for room i; and TF_i = estimated time fraction ıch individual spent in room i during the fall semester $(\text{TF}_{i \to k}$ sums to 1.0).

The IEI estimated from each individual factor was ·eated either as a dichotomous variable ('no' vs. 'any xposure'), or as a continuous variable for the AWSS. EIs estimated from the combinations were also treated s continuous variables. In estimating IEIs for the ccupants who spent some time in non-evaluated ɔoms in the newest building (in the comparison roup), a zero value was assigned to the exposure for ıat time fraction.

We used generalized linear regression models to xamine the association of AWSS with visible mold, ıold odor, and moisture and to obtain least squares ıean values (LSM) of AWSS (SAS Institute Inc., ;ary, NC, USA). To compare building-related respir-tory symptom prevalences between building groups, /e used chi-square tests. We examined the association f IEIs with building-related respiratory symptoms sing multivariate logistic regression models adjusting or age, gender, smoking, job status (faculty or staff), ear of hire, presence of allergies, and use of latex loves. We reported odds ratio (OR) and Wald's 95% onfidence interval (CI). We used Somers' D statistics SDS) of rank correlation to compare the predictive bility of the logistic models using the AWSS-based EIs to those using the combination-based IEIs. We xamined exposure–response relationships using quar-iles of the AWSS-based IEIs and the visible-mold-veighted combination-based IEIs by symptom in the ogistic models (SAS Institute Inc., Cary, NC, USA).

lesults

;ymptom prevalences

Among the 393 participants (mean age = 51, range = 5–65; 54% female; 87% white), approximately 30% eported at least one of the lower respiratory symp-oms, about 60% reported nasal and/or sinus symp-oms, and 40% reported throat irritation (Table 1). For each symptom, more than half of the symptomatic ɔarticipants reported building-relatedness. Of the 393 ɔarticipants, 43% reported asthma-like symptoms (wheeze, or awakened by shortness of breath, or chest tightness around animals or dust, or continuous breathing troubles) (Burney et al., 1989), and 18% reported hypersensitivity pneumonitis (HP)-like symptoms (at least one lower respiratory symptom such as wheezing, chest tightness, shortness of breath with exertion, or usual cough, and any systemic symptom such as fevers, chills, night sweats, or flu-like achyness) (Arnow et al., 1978; Fox et al., 1999; Hodgson et al., 2001). Seventeen percent of the participants reported physician-diagnosed asthma, and half of those asthmatics reported building-relatedness.

Table 1 Demographic characteristics and prevalence of respiratory symptoms within the past 12 months for all participants ($N = 393$)

Characteristics/symptoms	No. of people	Prevalence (%)
Demographics		
Average age in years (s.d.)	51 (10)	–
Gender		
Female	214	54
Male	173	44
Not responded	6	2
Ethnicity		
White	344	87
African-American	22	6
Others	14	4
Not responded	13	3
Smoking status		
Non-smokers	262	67
Ex-smokers	113	29
Current smokers	16	4
Not responded	2	<1
Respiratory symptoms		
Lower respiratory symptoms		
Wheezing	129	33
Chest tightness	105	27
Shortness of breath	136	35
Attack of cough	113	29
Upper respiratory symptoms		
Nasal symptoms	238	61
Sinus symptoms	234	60
Throat irritation	163	41

The prevalence of any building-related respiratory symptom was significantly higher ($P < 0.02$) in the water-damaged building group than in the comparison building group (≥20% vs. ≤ 7% for any lower respiratory symptom, and ≥34% vs. ≤ 17% for any upper respiratory symptom) (Figure 1).

Environmental evaluation

A higher prevalence of rooms with water stains, visible mold, mold odor, or moisture was observed in the water-damaged building group compared with the comparison building group (Table 2). The average AWSS of the rooms in the water-damaged buildings (0.80) was higher than that in the comparison buildings (0.38). The newest comparison building had an average

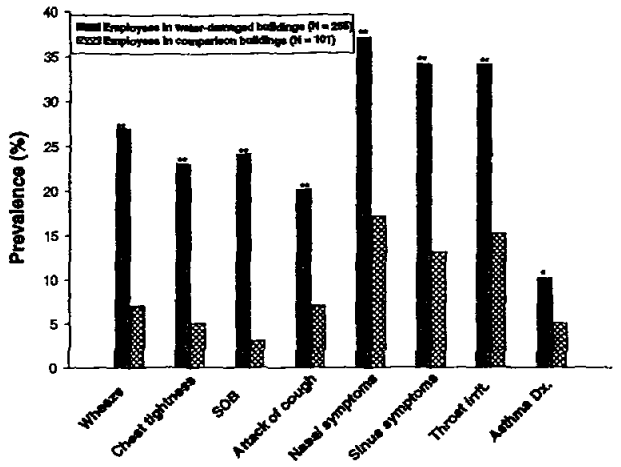

Fig. 1 Prevalence of building-related respiratory symptoms (wheeze, chest tightness, shortness of breath (SOB), attack of cough, nasal symptoms, sinus symptoms, throat irritation (throat irrit.), and physician-diagnosed asthma (asthma dx.) by building group. Only those who had responded to a question about the primary building they had been working in during school year were included in this analysis. A department in which the majority of faculty was relocated was excluded from the analysis (**$P < 0.02$; *$P = 0.14$ for chi-square test of two prevalences, adjusted for age, gender, smoking, job status, year of hire, allergies, and use of latex glove in models)

AWSS of 0.08, and only one of 52 rooms investigated had visible mold (data not shown).

In the multivariate analyses, water-damaged buildings had significantly higher ($P < 0.0001$) average AWSS (LSM = 0.81) than the comparison buildings (LSM = 0.44) after controlling for fixed effects of room type (classroom or office), floor, industrial hygiene team, temperature, and relative humidity. In logistic regression models controlling for room type, the water-damaged buildings showed significantly higher odds of visible mold (OR = 11.4; 95% CI = 4.0–31.9) and of mold odor (OR = 4.5; 95% CI = 1.1–19.3) compared with the comparison buildings.

Environmental factors were significantly correlated with one another. The average AWSSs were significantly higher ($P < 0.005$) in rooms with mold odor (0.99), visible mold (1.18), or damp/wet material (1.20) than in rooms without them (0.69, 0.62, 0.70, respectively). The odds of mold odor were four times higher (95% CI = 1.9–8.4) in rooms with visible mold than in rooms without visible mold.

Association of IEIs with respiratory symptoms

The AWSS-based IEIs for the 323 employees ranged from 0 to 2.2 (mean = 0.63, median = 0.65, and s.d. = 0.43). The combination-based IEIs ranged from 0 to 1.8 (mean = 0.89, median = 1.0, and s.d. = 0.45) for the water-stain-weighted combination and from 0 to 1.6 (mean = 0.63, median = 0.5, and s.d. = 0.42) for the visible-mold-weighted combination. The IEIs based on AWSS or visible mold were more consistent predictors for building-related respiratory symptoms compared with IEIs based on mold odor or moisture (Table 3). In multivariate logistic models the AWSS-based IEI was a significant predictor for building-related wheeze (OR = 2.3) and throat irritation (OR = 2.4). Exposure to visible mold was associated with significantly increased building-related wheeze (OR = 2.0), chest tightness (OR = 2.6), shortness of breath (OR = 2.6), nasal symptoms (OR = 1.7), and sinus symptoms (OR = 2.0). Exposure to mold odor was associated with significantly increased throat irritation (OR = 2.3). We also found significant associations of the combination-based IEIs with building-related chest tightness, shortness of breath, nasal symptoms, and sinus symptoms (range of ORs = 2.2–2.7). We did not find significant associations of any of these exposure indices with physician-diagnosed asthma reported as building related. The odds of building-related wheeze, shortness of breath, nasal symptoms, and throat irritation generally increased as IEI increased in quartile analysis (Figure 2). Similar trends were also observed for other symptoms (not shown).

The AWSS-based IEI model appeared to be better in predicting wheeze and throat irritation than either of the combination-based IEI logistic models (SDS: 0.42 vs. 0.38, 0.39 for wheeze; 0.32 vs. 0.28, 0.29 for throat irritation). However, these models showed similar predictive abilities for all other building-related respiratory symptoms. The logistic model using the water-stain-weighted combination IEIs better predicted building-related nasal symptoms

Table 2 Distribution of observational measurements for rooms by building group

		Water stains			Any mold odor *N* (%)		Moisture condition *N* (%)	
Building group[a]	No. of rooms	Continuous Mean (s.d.)	Any *N* (%)	Any visible mold *N* (%)	Slight	Strong	Damp	Wet
Water-damaged buildings	558	0.80 (0.5)	549 (98)	109 (20)	23 (4)	7 (1)	4 (<1)	4 (<1)
Comparison buildings	163	0.38 (0.4)	122 (75)	4 (3)	2 (1)	0 (0)	0 (0)	0 (0)
Total	721	0.71 (0.5)	671 (93)	113 (16)	25 (3)	7 (<1)	4 (<1)	4 (<1)

[a] Water-damaged buildings: buildings that had a recurrent history of water incursion and related renovations; comparison buildings: buildings that were reported to have had little or no problems with water incursion.

ıble 3 Adjusted[a] odds ratios (95% CIs) of respiratory symptoms for exposure indices from each environmental factor and from linear combinations of the environmental factors

	Water stains					Factor combinations	
ıilding-related ıspiratory symptoms	Continuous variable	Any stains	*Any visible* mold	Any mold odor	*Any damp material or* standing water	Water-stain-weighted[b]	Visible-mold-weighted[c]
ıwer respiratory symptoms							
Wheeze	**2.3** (1.1–4.5)	2.6 (0.7–9.2)	**2.0** (1.1–3.7)	1.1 (0.5–2.3)	1.2 (0.3–4.5)	1.8 (0.9–3.5)	1.7 (0.9–3.4)
Chest tightness	1.9 (0.9–3.8)	1.9 (0.5–6.9)	**2.6** (1.3–4.9)	1.01 (0.5–2.2)	1.0 (0.2–4.2)	1.8 (0.9–3.8)	**2.2** (1.1–4.6)
Shortness of breath	1.7 (0.8–3.6)	6.3 (0.8–51.1)	**2.6** (1.3–5.1)	1.4 (0.7–3.2)	3.3 (0.9–11.9)	**2.7** (1.2–6.1)	**2.5** (1.2–5.4)
Cough	1.3 (0.6–2.6)	3.2 (0.7–14.4)	1.5 (0.8–2.8)	1.7 (0.8–3.6)	1.0 (0.2–4.5)	1.5 (0.7–3.2)	1.7 (0.8–3.6)
pper respiratory symptoms							
Nasal	1.5 (0.8–2.8)	**4.4** (1.2–15.3)	**1.7** (1.0–3.0)	1.1 (0.6–2.1)	1.7 (0.5–6.0)	**2.4** (1.3–4.6)	**2.5** (1.3–4.7)
Sinus	1.6 (0.9–2.9)	**3.8** (1.1–13.4)	**2.0** (1.2–3.4)	1.3 (0.7–2.5)	0.8 (0.2–2.9)	1.8 (1.0–3.4)	**2.2** (1.2–4.1)
Throat irritation	**2.4** (1.3–4.4)	2.0 (0.7–5.6)	1.3 (0.7–2.1)	**2.3** (1.2–4.3)	1.5 (0.4–5.1)	1.6 (0.9–3.0)	1.5 (0.8–2.8)

Adjusted for age, gender, smoking, job status, year of hire, allergies, and use of latex glove in logistic regression models Boldfaced odds ratios are statistically significant ($P < 0.05$)
Water-stain-weighted index: 1 (if water stains) + 0.5 (if visible mold) + 0.5 (if mold odor) + 0.5 (if damp or wet).
Visible-mold-weighted index: 0.5 (if water stains) + 1 (if visible mold) + 0.5 (if mold odor) + 0.5 (if damp or wet).

han that using only the AWSS-based IEIs (SDS:).41 vs. 0.37). Both the combination-based and the AWSS-based IEI models did best predicting building-elated shortness of breath (SDS: 0.48–0.50) compared with other building-related respiratory symptoms (SDS: 0.28–0.43).

lelocation and non-respondent surveys

To supplement the associations between dampness/mold exposure indices and building-related respiratory symptoms, we examined the effect of employee relocation from a water-damaged building to comparison buildings. Of the 26 relocated employees, 14 employees (54%) reported building-related respiratory symptoms while they worked at the water-damaged building. Of these 14, 36% reported that their symptoms had either lessened or completely resolved after the relocation.

In the non-respondent survey, the major reasons given for non-response in the main study were unrelated to health. The only demographic difference between respondents and non-respondents was a higher proportion of males among non-respondents (56% vs. 44%). The prevalence of physician-diagnosed asthma was almost identical between respondents (17%) and non-respondents (16%), although respondents were more likely to have any chest symptom than non-respondents (46% vs. 30%). Non-respondents in the water-damaged building group had increased symptom prevalence compared with non-respondents in the comparison building group, as was found for respondents.

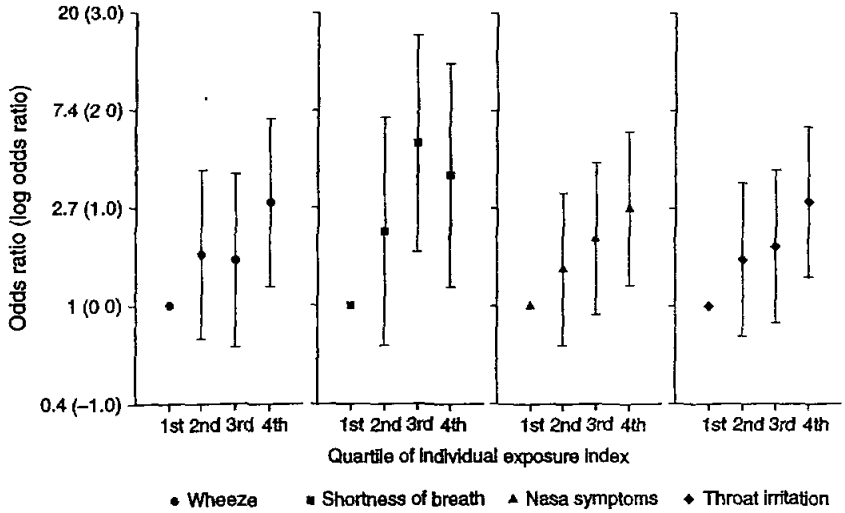

Fig. 2 Exposure–response relationships across quartiles of individual exposure indices (IEIs) and work-related respiratory symptoms. Quartiles based on AWSS-based IEIs for wheeze and nasal symptoms; quartiles based on the visible mold-weighted combination IEI for shortness of breath and throat irritation. Similar trends were also observed for other symptoms (not shown)

Discussion

Building-related respiratory symptoms and diagnoses

Three pieces of evidence combine to suggest a relationship between building conditions and symptom prevalence in our study. First, the distribution of symptoms and physician diagnoses across the two building populations was uneven. In this, the prevalences of building-related respiratory symptoms and damp conditions were both considerably higher in the water-damaged building group than in the comparison building group, suggesting that occupants' symptoms may have resulted from building conditions. Secondly, improvement or resolution of symptoms among a portion of employees relocated from a water-damaged building to other buildings suggests that their symptoms may have been caused by exposure in the water-damaged building. Thirdly, and most important, our statistical models demonstrated not only associations but also exposure–response relationships between semi-quantitative dampness/mold exposure indices and building-related respiratory symptoms.

Consistent with building-related respiratory disease, the college employees in the water-damaged building group had an excess of symptoms and physician-diagnosed asthma in comparison with two external groups. First, compared with occupants of US office buildings not known to have indoor air quality complaints, the college employees had higher prevalences of lower respiratory symptoms (>27% vs. <6%) and upper respiratory symptoms (>41% vs. <14%) (Apte et al., 2000). Secondly, the college employees also reported more physician-diagnosed asthma (18%) than adult residents in the same state (11%) (National Center for Chronic Disease Prevention and Health Promotion, 2001).

Building-related asthma is characterized by chest tightness, wheezing, cough, and shortness of breath with one of several patterns of exacerbation related to building occupancy. Building-related HP is characterized by chest symptoms often accompanied by fevers, chills, or flu-like achyness related to building occupancy. Likewise, building-related allergic rhinitis is suspected when building occupants have nasal symptoms and sneezing in relation to building occupancy (Kreiss, 1989). In our study, many persons with asthma- or HP-compatible symptoms did not report a physician diagnosis of either. Other investigators have reported that over 70% of those with asthma symptoms and airflow obstruction in the general population are not diagnosed with asthma (van Schayck et al., 2000). Similarly, in outbreaks of HP and other similar granulomatous lung diseases, symptomatic cases may not be diagnosed as such by physicians (Rose et al., 1998; Sanderson et al., 1992). Therefore, the symptoms reported in our study may reflect the presence of building-related diseases even among those not specifically diagnosed.

Semi-quantitative dampness/mold exposure indices

Our study shows that semi-quantitative dampness/mold exposure indices created from visual and olfactory observations predicted building-related respiratory symptoms in 13 buildings on this college campus. In our study, trained industrial hygienists assessed room environments rather than relying on occupant-reported exposures, which are subject to information bias. Many studies have reported the association of damp environments with respiratory health using dichotomous assessment of exposure (Andriessen et al., 1998; Brunekreef, 1992; Dales et al., 1991b; Jaakkola et al., 2002). To minimize bias due to an arbitrary choice of dichotomous exposure criteria (Bornehag et al., 2001), we developed a continuous exposure scale using a time-weighted semi-quantitative assessment tool for dampness/mold. By treating a semi-quantitative scale as a continuous variable in the statistical models, we could examine exposure–response relationships.

Haverinen et al. (2001) showed that a three-level classification of water-damage for entire residential buildings, using both the amount of moisture damage and its severity, better predicted health symptoms than a two-level (no/minor vs. moderate/severe water damage) classification. They demonstrated exposure–response relationships using the three-level classification. Our evaluation for water stains was a four-level classification based on the percentage of area stained in seven different areas in each room and all our IEIs were weighted for time spent in particular rooms, yielding continuous variables for statistical modeling. Our quartile analyses of continuous IEIs showed that an increasing exposure to dampness/mold was generally associated with elevated odds of building-related respiratory symptoms.

Our observation of exposure–response relationships for several building-related respiratory symptoms suggests that our exposure indices may be valid surrogates for the related exposure(s). Although the specific microbial cause(s) remains unknown, a robust body of knowledge exists to support the association of moisture incursion in residences with risk of asthma and respiratory symptoms (Andriessen et al., 1998; Brunekreef, 1992; Dales et al., 1991a; Haverinen et al., 2001). Our study adds to the existing evidence indicating that the same risks occur in water-damaged nonresidential buildings (Jarvis and Morey, 2001; Li et al., 1997; Savilahti et al., 2000; Wan and Li, 1999). Dales et al. (1997) showed that self-reported mold odor was associated with total culturable fungi in settled dust, and visible mold growth in homes was associated with increased levels of *Aspergillus* and *Penicillium* in settled

semi-quantitative dampness/mold exposure indices, weighted by time spent in specific rooms and based solely on visual and olfactory observation, were associated with building-related symptoms that may reflect asthma, HP, and nasal/sinus disease. Our findings demonstrate the usefulness of the observational semi-quantitative approach for exposure assessment in large epidemiologic studies. Our semi-quantitative method suggests that not only is the presence of water stains, visible mold, mold odor, or moisture important for predicting building-related respiratory diseases, but also that the relative extent of these factors can be used to prioritize remediation to reduce potential risk of building-related respiratory diseases. From a public health perspective, these observational findings justify action to correct water leaks and repair water damage in order to prevent building-related respiratory diseases. Although not addressed by our study, hidden reservoirs of microbial contamination should not be ignored in remediation of water-damaged buildings.

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Characterization of Mold and Moisture Indicators in the Home

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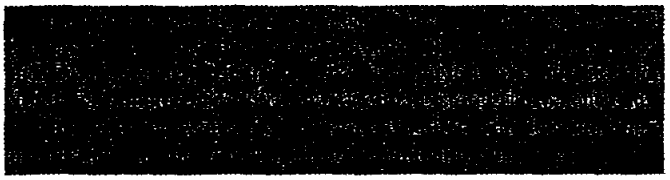
As studies increasingly support the presence of health risks associated with mold and moisture, understanding fungal concentrations and physical measurements as they relate to the microenvironment becomes more important. We conducted a cross-sectional study in the homes of 64 subjects. The primary objective of this study was to use trained inspectors' list of indicators in rooms (bathroom, bedroom, and basement) and determine whether these indicators are associated with higher fungal levels or physical measurements. A new category for combining the concentrations of fungal species, referred to as moisture indicator fungi (MIF), is used in the analysis. Our results show that basements with a musty odor, efflorescence, water sources, or mold have a two- to threefold increase in fungal concentrations over basements without these indicators. The regression model for the basement was highly predictive of indoor MIF concentrations ($r^2 = .446$, $p = .017$). Basement water sources are substantial predictors of indoor total fungi, MIF, and Aspergillus/Penicillium *spp. MIF concentrations are higher in homes with basement water sources, and most notably, the increase in MIF concentrations is significant in other living spaces (bathroom and bedroom) of the dwelling. Basement water sources are important moisture/mold indicators for epidemiologists to use in exposure assessments performed in residential dwellings.*

Keywords basements, exposure, indoor, moisture, mold, residential



The health risks associated with moisture indicators in naturally ventilated buildings and the potential for indoor microbiological exposures are well documented in a recent literature review.[1] Studies linking moisture and mold in buildings to health suggest that the association becomes stronger as the reliability of moisture damage and bioaerosol assessment techniques improve.[2–4] The U.S. National Academy of Sciences states that there is "sufficient evidence of an association between fungal exposure and symptom exacerbation in sensitized asthmatics."[5,p xx] Other studies suggest that moisture in the home is associated not only with asthma but also with sarcoidosis[6] and usual interstitial pneumonitis.[7,8]

According to the 1999 American Housing Survey, 10% of homes surveyed report water leaks.[9] Water problems include leaks from inside as a result of faulty pipes or plumbing fixtures and outside sources (poor drainage, leaky windows, and faulty exterior cladding). In U.S. housing, a higher prevalence of water leaks is reported from sources outside the structure.[9]

In 1993, the U.S. Institute of Medicine reviewed the mechanisms by which moisture enters the built environment and identified weaknesses in building assessment protocols, measurement techniques, and the various standards that control or regulate moisture.[10] The report explained that a high rate of moisture may enter the indoor built environment from moist soil that is often associated with drainage around foundations. In addition, basements—spaces that are usually below grade, cool, damp, poorly ventilated, and near water tables—are more likely to act as reservoirs of microbial growth than any other part of a building.[11,12]

Several studies have examined the aspects of moisture that are associated with biological contamination; these include exhausts in kitchens and bathrooms; below grade moisture seepage; bulk water (plumbing leaks, roof drainage, and envelope penetration); condensation on inadequately insulated outside walls; and inappropriately sized cooling coils (i.e., incorrect latent heat ratio).[13–18]

In many of the epidemiologic studies showing an association between moisture and adverse respiratory health effects or lung disease, exposure is often defined with both qualitative

and quantitative methods. Exposure assessment methods used to characterize moisture and mold include the following: (1) physical measurements (e.g., humidity, temperature); (2) sampling and analysis to detect microbes indicative of moisture; (3) visual inspections for moisture and mold; or (4) questionnaires (self-reports or interviewer-administered).[19] Douwes et al.[20] identified the limitations in bioaerosol exposure assessments from a quantitative perspective.

Cooper-Arnold et al.[3] reviewed moisture and the association with pulmonary disease in 15 studies to define a best estimate of population attributable risk (PAR). They found that the method of exposure assessment influences the statistical significance of apparent relationships between moisture indicators and lung disease. Studies using physical or visual measurements reported higher odds ratios than self-reported questionnaires. Five studies were identified as "best studies" based on exposure assessment technique (defined as using field inspection and bioaerosol sampling), and the authors reported a PAR of 24.3% for airways and pulmonary disease.

As studies increasingly support the presence of health risks associated with mold and moisture, understanding fungal concentrations and physical measurements as they relate to the microenvironment becomes more important. Reports of damp spots, water leakage, or water damage, and observations of mold or mildew from self-report questionnaires, are used as surrogate measures for the number of fungi in several published epidemiologic studies.[21–26] Several studies have relied on home inspections for verifying moisture and mold in the home.[2,25,27–30] Haverninen[2] defined three levels of criteria for the severity and amount of moisture damage in a home for inspectors to use as an indirect method of mold exposure and demonstrated dose-dependent risk increases for several health symptoms.

Dharmage,[31] Garrett,[32] Su,[33] and Waegemaker[30] measured the presence of fungal propagules in air and demonstrated that reported house characteristics, such as visible mold or dampness patches, have validity as measures of mold concentrations and dampness in homes. Dharmage[31] showed that higher total airborne fungal concentrations were associated with visible mold. Garrett[32] found significantly higher *Cladosporium* concentrations in homes with substantial visible mold (998 CFU/m^3) compared with homes with none to slight (458 CFU/m^3). After conducting a factor analysis, Su[33] reported that elevated airborne concentrations of total soil fungi collected in the living room or family room were significantly associated with the dirt floor, crawl-space type of basement.

Waegemaeker[30] used three kinds of criteria to define dry and damp in a pilot study of 36 homes and found that viable fungal spore concentrations were higher in damp homes than dry homes. Other studies, such as Ren[34] and Verhoeff,[29] found only a weak relationship between house characteristics, as described by a questionnaire, and the presence of fungal propagules in indoor air.

Fungal exposure and its association with moisture damage in a building is complex and multifaceted. Many types of fungal species are reported to grow in the indoor home environment.[35–37] Fungal indicator species that relate to moisture are described in the literature.[38,39] One way to provide a better understanding of the influence of residential characteristics on fungal levels is to clarify the definitions of "fungal levels." Most studies use total airborne fungi concentrations or report a dominant type of fungi species, such as *Cladosporium* or *Aspergillus*, in their exposure assessment analyses. Li and Kendrick[40] found signficantly higher airborne fungal spore counts of specific species (*Aspergillus/Penicillium*, *Cladosporium*, unidentified basidiospores, etc.) in damp residences (defined as homes with visible mold, water damage, or water in the basement).

Fungi can grow only on a surface or in a substrate. Many conditions of the surrounding environment (e.g., relative humidity and temperature) affect fungal growth by increasing or decreasing the drying potential of the substrate. In general, water requirements for fungi are species specific. For example, *Chaetomium globosum*, *Ulocladium botrytis*, and *Stachybotrys chartarum* thrive on wet substrates, whereas some *Aspergillus* spp. (such as *A. penicillioides* and *A. restrictus*), and *Eurotium* spp. (such as *E. amstelodami* and *E. repens*) prefer substrates that have less available free water.[35,41] Exposure assessments may prove more useful if a broad group of fungal species is selected according to their nutrient requirements and substrate characteristics, including water availability.

The goal of this study was to improve the characterization of commonly used moisture/mold indicators, such as observations of mold, musty odors, absence of exhaust fans, and reports of water sources in residential dwellings. Several important moisture/mold indicators—defined in this study as an observed condition that may contribute, cause, or result from water vapor or free water—were identified in specific rooms of a dwelling.

METHODS AND MATERIALS

Study Design

This study was part of a larger case-control study exploring the relationship between moisture and mold and interstitial lung disease (ILD). A cross-sectional sampling strategy was employed in the homes of subjects. Several different exposure assessments of moisture and mold were used in the home including self-report questionnaires, walkthrough evaluations by an inspector using a checklist, physical measurement sampling (temperature and relative humidity), and airborne fungal sampling.

Sampling goals included collecting fungal samples and physical measurements outdoors and in three different rooms of a home. Rooms, such as the basement and bathroom, were specifically targeted based on their likelihood to have moisture or fungal growth present in the area. Bedrooms were also targeted because they are where individuals sleep and spend most of their time when indoors. Specific information collected from subjects (self-report questionnaires) and trained inspectors (walkthrough evaluations) regarding house characteristics is compared.

The primary objective of this study was to use the inspectors' list of moisture indicators in a room and determine whether these indicators were associated with higher fungal levels or physical measurements. Airborne concentrations of a unique group of culturable fungal species, referred to as moisture indicator fungi (MIF), are described in this article.

Subject Recruitment

Study subjects with ILD (cases) were obtained from a pathology registry at Hartford Hospital, selected from physicians throughout central Connecticut. Subjects without ILD (controls) were identified from a random digit-dialed list generated by the Roper Center at the University of Connecticut, Storrs, Conn., as part of Tier I for population surveys. Fifty-three cases and 50 controls were eligible to participate in the case control study. In 1997, study subjects were mailed a 40-item questionnaire, referred to as self-report questionnaire (SRQ), addressing moisture and aerosolization in their home and work environment.

The survey instrument used for this study was adapted from Mullen[7] and Ortiz.[6] The survey instrument was modified to expand on the questions related to moisture and mold. For example, the question "Does your basement have water problems?" was added. Another question regarding the presence or absence of carpeting in the home was modified to identify the location of the carpet in the home (e.g., bedroom or living room).

Participants were asked about their occupational histories, allergies, hobbies, smoking status, and household characteristics. Approximately 21 items were related to household characteristics, including type of home, heat source, humidifier use, carpet status, water problems, visible mold growth, and presence of exhaust fan in kitchen and bathrooms. Once the SRQ was returned and subjects granted permission, trained inspectors visited and conducted a walkthrough evaluation of each dwelling.

Investigators assessed 64 residences (30 cases and 34 controls) in this phase of the study. The time period between the returned self-reports and walkthroughs was 3–6 months. Before each assessment, subjects were asked not to clean and to keep their windows closed at least an hour before the site visit (data regarding compliance was not collected). All procedures involving human subjects were reviewed and approved by the University of Connecticut Health Center Institutional Review Board before the study began.

Walkthrough Evaluations

The majority of homes recruited for the study were located in central Connecticut. Two inspectors were trained to use the same protocol for each home visit. Walkthrough evaluations were performed primarily in the spring, summer, and autumn between the years 1997 and 1999. The trained inspectors used a 45-item checklist, referred to as the inspector-rated checklist (IRC), designed by the investigators after a thorough review of the literature for positive associations between self-reported dampness in homes and health effects. Trained inspectors used the IRC to identify the presence or absence of moisture/mold indicators in specified rooms in the home.

The information on the IRC includes general housing characteristics such as design and age of the dwelling, types of heating, type of flooring, ventilation, humidifier use, and number of houseplants. In addition, the IRC focuses on specific room observations for "dampness," including the absence of exhaust fans in bathrooms, visible mold, evidence of moisture damage (stained ceiling tiles), and several water-related basement observations (spalling, wall efflorescence, musty odor). Observations of each dwelling were made using the IRC in the kitchen, living room, bedrooms, bathrooms, laundry room, and basement. In this study, the analysis used the items on the IRC related to the bathrooms, basements, and bedrooms.

Trained inspectors asked occupants about the dwelling, such as history of water damage in the home, because it was not possible to obtain this information by observation alone. The inspectors also took a photograph of each home and its surrounding ground. To assess the contribution of moisture load as it relates to surface drainage around a home, co-author Turner developed a building drainage score for each house. This author used a photo, a house footprint, and his years of experience with moisture intrusion in basements based on surface grading of the earth to assign one of the three scores as a way to describe the potential for surface drainage to contribute to moisture load: 1 = little potential, 2 = more potential, and 3 = lots of potential.

Sampling Rooms

Sampling was conducted on the same day as the walkthrough evaluation by the inspector. One outdoor and three indoor sampling sites were chosen at each dwelling. Complete sampling was conducted in 63 homes; two samples were not collected from one sampling site in one home. The criteria for the first sampling location in the home included a room with the following characteristics: a history of flooding or water leaks; visual evidence of water damage; or a room prone to moisture incursion (the basement, if present, or a bathroom). Once this initial "wet" room was chosen, another room having the next greatest potential for moisture incursion or damage was chosen. The bedroom was selected because it was considered the place where the subject spent the most time when at home. The three most commonly sampled indoor locations were the bathroom, the basement, and the subject's bedroom. In 77% of the homes, the basement was chosen as the first "wet" room, using the criteria as defined in the methods.

Descriptive statistics of household demographics as reported on the IRC were completed for all residential dwellings. During the site visit, inspectors originally used five indicators in the basement, four in the bathroom, and five in the bedroom from the IRC. Some of these indicators were collapsed to create a composite indicator for analysis purposes. In the basement, the moisture/mold indicators were: musty odor, wall efflourescence, water sources, visible mold, and spalling. In

TABLE I. Mold/Moisture Indicators Used by Inspectors During Home Walkthrough Evaluations

Mold and Moisture Indicator	Definition
Bathroom	
Absent exhaust fan	The bathroom fan is not present or is not functional. It is not externally exhausted to the outside.
Visible moisture damage	The presence of water damage. For example, the presence of water stains on ceiling, walls, or around the windows. Bubbling paint on windowsill or other area.
Visible mold in shower	The visible presence of fungal colonies of any color on surfaces, including tiles, walls, and ceilings in the tub/shower enclosure.
Visible mold in other	The visible presence of fungal colonies of any color on surfaces, including tiles, sinks, walls, ceilings, toilet, windows, or floors in other areas of the bathroom excluding the tub/shower enclosure.
Basement	
Water sources	Any information or signs related to leaks, defective drainage, water accidents, uncontrolled water activity, or water intrusion events in the basement at any period during the occupant's time of living in the dwelling and/or during the life of the dwelling.
Wall efflorescence	The presence of "crystals" or white markings on the walls in the basement.
Spalling	The breaking off or bursting out of surface layer of concrete or brickwork, usually caused by freezing of intruded water. This is often present as holes, divots, or surface defects.
Musty odor	A damp, musty smell is noticed when entering the room.
Visible mold	The visible presence of fungal colonies of any color on surfaces, walls, ceilings, windows, or floors, most often small colonies on surfaces without.
Bedroom	
Visible moisture damage	The presence of water damage. The presence of a water stains on the ceiling, walls, or around the windows. Bubbling paint on windowsill or other area.
Visible mold	The visible presence of fungal colonies of any color on surfaces, walls, ceilings, windows, or floors.
Wall-to-wall carpet	Carpeting is present from wall to wall in the room.

the bathroom, indicators were: absent exhaust fan, mold in shower, water or moisture damage, and mold in a location other than shower. Another bathroom mold indicator was created by collapsing two mold bathroom indicators (mold in shower and mold in other) into one, referred to as "mold any."

The bedroom indicators included wall-to-wall carpeting, visible moisture damage, visible moisture damage on ceiling, visible moisture damage on windows, and visible mold growth. Carpeting was included in the analysis because of its potential to act as a reservoir of microbial growth. Because 85% of the data for bedroom visible moisture damage on ceiling and on windows were missing from the homes, these two moisture indicators were not explored. Only three homes had evidence of spalling in the basement and mold in the bedroom; therefore, descriptive statistics for these indicators were not included. In summary, a total of 11 mold and moisture indicators were analyzed in combination with the environmental and fungal sampling in this study.

Fungal Sampling

Quiescent fungal airborne sampling was conducted in each room.[19] The sampler was placed on paper on the floor during sampling. Samples were collected in each dwelling using an Andersen N-6 single stage sampler (Graseby Andersen, Atlanta, Ga.) for 1 to 2 min. Samplers were calibrated annually by the manufacturer and operated at a flow rate of 28.3 L/min. All sampling was performed in duplicate. Samples were collected on 2% malt extract agar (20 g DIFCO malt extract broth, Becton Dickinson, Sparks, Md.; 20 g agar/L distilled water). Sixty-four duplicate indoor samples were collected in a basement, bedroom, and bathroom, respectively. In total, 382 indoor samples (2 samples were not collected from 1 sampling site in a case home) and 64 outdoor samples were collected.

Samples were sent to the laboratory by overnight mail on the day they were collected. They were incubated at 25°C on receipt by the laboratory. Two readings, including counting and identification, were performed on the seventh and tenth days after collection. All fungal colonies were identified and counted. Dominant species of *Aspergillus* and *Penicillium* were identified and recorded in the data analysis as one pooled taxon.[36,40] Fungal concentrations are presented in CFU/m^3. The limit of quantification was 35 CFU/m^3 for 1-min samples and 18 CFU/m^3 for 2-min samples. Concentrations of the paired duplicate samples were averaged, and a mean concentration in CFU/m^3 was used in the analyses.

Because of the large number of biological sample comparisons, a hierarchy of coding criteria was established beforehand based on the investigators' experience and views. After

TABLE II. Moisture Indicator Fungi (MIF) List

Fungi Species		
Acremonium spp.	Basidiomycetes	*Phoma* spp.
Alternaria alternata	*Chaetomium globosom*	*Rhizopus stolonifor*
Aspergillus candidus	*Cladosporium* spp.	*Rhodotorula* spp.
A. fumigatus	*Epicoccum nigrum*	*Sporobolomyces* spp.
A. niger	*Eurotium amstelodami*	*Stachybotrys chartarum*
A. ochraceus	*E. rubrum*	*Trichoderma harzianum*
A. versicolor	*E. repens*	*Trichoderma koningii*
A. sydowii	*Exophiala* spp.	*Tritirachium oryzae*
A. penicillioides	*Fusarium* spp.	*Ulocladium chartarum*
A. restrictus	*Mucor* spp.	*Wallemia sebi*
A. sclerotiorum	*Paecilomyces* spp.	
A. ustus	*Penicillium* spp.	
Aureobasidium pullulans		

Note: Several factors, such as fungal water activities, nutrient requirements, and fungal spore discharge mechanisms, played a key role in defining the criteria for establishing MIF.

total fungal counts (TOT) were calculated, fungal species were grouped into three subcategories: (1) MIF, (2) *Aspergillus/Penicillium* spp. (A/P), and (3) all other fungi. The MIF grouping (Table II) was constructed by co-author Yang by using fungal characteristics, such as fungal water activity[41–43] and fungal spore mechanisms.[44–47] The primary factor (i.e., fungal water activities) played a key role in defining the criteria for MIF. For example, some fungi that grow at high water activity conditions are called hydrophiles. Species of *Acremonium*, *Chaetomium*, *Stachybotrys*, and others, require high water activity and thrive on wet substrates.

Another group of fungi are xerophilic and grow at low water activity. Some common xerophilic fungi species found indoors are *Eurotium*, *Aspergillus restrictus*, *A. penicillioides*, and *Wallemia sebi*. Their detection and growth prefer low water activity substrates. These fungi are often found on substrates subjected to high humidity and poor ventilation but generally not wet conditions. There is a third group, in between the hydrophiles and the xerophiles, that are more commonly found on water-damaged materials. They are called xerotolerant fungi and include *Aspergillus versicolor* and *A. sydowii*, and others.

Concentrations of each taxa in the MIF category were totaled and reported in CFU/m^3. *Aspergillus* spp. (several species are known to be pathogens) and *Penicillium* spp. are two of the most frequently found fungi indoors, especially in problem buildings.[37] *Aspergillus* or *Penicillium* spp. may also be regarded as indicator taxa, defined usually as a particular fungus that carries an excess health risk or presents a warning of environmental damage.[19,48]

Physical Sampling

Physical measurements, including dry bulb temperature, dew point temperature, and relative humidity, were taken simultaneously with the fungal sampling at three locations in each dwelling. Temperature and relative humidity measurements were taken at each indoor site with a Velocicalc Plus 8360 (TSI Inc., St. Paul, Minn.). Physical measurements provide basic information about the amount of moisture in the air and/or in building material. Although the study design originally incorporated the use of indirect water content measures (conductivity using a Tramex moisture meter [model; Tramex, Littleton, Colo.] or a Delmhorst BD 2000 moisture meter (Delmhorst, Towaco, N.J.), these were found not to be practical in the course of the study.

Data Analysis

All data were maintained in ACCESS version 9.0 and analyzed in SPSS version 10.0 in a Windows® environment. A subset of approximately 15 questions from the SRQ and IRC were analyzed to determine the relationship between moisture assessment performed in the home by occupants and trained inspectors. Housing questions ranged from general items such as type of heating to more specific dampness items such as musty odor in the basement. Kappa statistics were used to measure the level of agreement in housing characteristics between the two types of data collection (self-report vs. inspection), with kappa values of 0.75 indicating excellent agreement and values below 0.4 indicating poor agreement.[49] At statistic with its associated probability (approximate significance) was used to test that each measure differed significantly from 0 and p-values.

The frequency distributions of the physical measurements (relative humidity, dew point temperature, and dry bulb temperature) were normally distributed, and parametric tests (independent sample t-test) were used to analyze the data. Central tendencies are represented as means (with standard deviations) in Table IV.

The frequency distributions of the fungal concentrations were skewed to the right. Log_{10} transformation yielded normal distributions, so central tendencies of the data are represented as geometric means (with 95% confidence intervals). Statistical analyses are conducted using parametric tests with log_{10}-transformed data. Three fungal groups were used in the analyses (TOT, MIF, and A/P).

Indoor-outdoor ratios of fungal measurements were calculated by dividing the indoor measurement of each room by the outdoor measurement of the home. Indoor-outdoor ratios are reported for three fungal categories (TOT, MIF, and A/P). These ratios were used to determine whether fungal amplification was evident in the room. An indoor-outdoor fungal ratio greater than one may indicate fungal amplification.[18] The frequency distributions of the indoor-outdoor fungal ratios were skewed to the right. Log_{10} transformation yielded normal distributions, so central tendencies of the data are represented as geometric means. Parametric tests (independent sample t-test) were used to analyze the data. Other descriptive statistics are

TABLE III. Relationship Between Self-Report Questionnaire (SRQ) and Inspector-Rated Checklist (IRC) Variables

Housing Characteristics	Kappa Statistic (n)[A]	p-value[B]
What year was your current home built?	0.91 (57)	<.0001
How long have you lived in your current home?	0.72 (62)	<.0001
How many houseplants do you have in your home?	0.65 (49)	<.0001
What kind of heat source do you use in your current furnace or boiler (oil, gas, electric)?	0.88 (64)	<.0001
What kind of heat distribution system does your current home have (forced air, hot water, gravity)?	0.90 (60)	<.0001
Do you use local humidifiers in your current home?	0.71 (59)	<.0001
In your current home, do you have wall-to-wall carpets in your bedroom?	0.87 (64)	<.0001
In your current home, do you have wall-to-wall carpets in your living room?	0.83 (63)	<.0001
Is air from your kitchen exhausted to the outside from a range hood?	0.59 (61)	<.0001
Is air from your bathroom exhausted to the outside?	0.53 (61)	<.0001
Is there mold growth present anywhere in the bathroom (in shower or on walls)?	0.22 (62)	.055
Does your basement have a musty or moldy odor?	0.21 (58)	.066
Does your basement have a water problem?	0.24 (56)	.022

[A] n = number of homes.
[B] A t statistic with its associated probability (approximate significance) was used to test that each measure differs significantly from 0. By using the kappa statistic as a measure of reproducibility, the p-values indicate the significant reproducibility between the SRQ and IRC.

provided (arithmetic mean, standard deviation, and range) to compare with values reported in the scientific literature.

Homes were classified into two groups according to the presence or absence of a moisture/mold indicator (as observed by inspectors). The independent sample t-test was used to test for significant differences in fungal levels or physical measurements in rooms with or without each moisture/mold indicator. Three sequential regressions were conducted to determine the presence of a relationship between fungal concentrations, physical measurements, and moisture/mold indicators. Fungal indoor measurements were used as the dependent variable. Each of the fungal categories (TOT, MIF, and A/P) was used separately as a dependent variable. Moisture/mold indicators of each room were the independent variables. Adjustments for season were conducted by using the outdoor concentrations as an independent variable in the model. Relative humidity was also used in the model as an independent variable.

The order of the three steps of the regression model included: (1) outdoor concentrations, (2) indoor relative humidity measurements, and (3) moisture/mold indicators of each room (indicators were added as a group block for each room). Stepwise regression analyses are described with Pearson correlations (r) and model summary r^2, standardized beta coefficients (β), and p-values.

RESULTS

Sixty-four homes (30 cases and 34 controls) were evaluated in this study. Fungal and physical measurements were collected in 64 homes and 191 rooms (1 room sample was not collected). Homes were sampled throughout different seasons, however, the majority of sampling was performed in the spring and summer.

Observations Between SRQ and IRC

Table III presents the relationship between SRQ and IRC observations. Basic environmental indicators, including age of dwelling, carpeting, and heat source, were in agreement between the self-report and the inspection. A formal comparison of the two assessments using kappa statistics suggests good agreement. However, home occupants were less likely to note mold or musty odors in the basement, mold growth in the bathroom, and problems of water in the basement than trained inspectors (kappa statistics < 0.3). Information collected on the IRC was used in further analyses for this article.

General Housing Characteristics

The majority of the homes were single-family dwellings located in central Connecticut. Approximately 12% were either apartments or condominiums. Subjects lived in the homes for approximately 13 years (range 2–24). The average number of inhabitants in the home was three. The mean size of the homes was 1825.4 ft^2. The average age of homes in this study was 46 years (range 12–80). The average age of the roof was 10 years (range 2–18). Sixty-four percent of the homes used oil as their heating source. The source of heat was distributed mainly by either hot water (52%) or forced air (44%). Approximately two-thirds of the homes had air conditioning. One-third of the homes used humidifiers. Thirty-eight percent of the homes used a dehumidifier in the basement. Pets were observed in 58% of the homes (14 homes with cats, 17 with dogs, 1 with a rabbit, 1 with a bird, and 4 with fish).

TABLE VII. Descriptive Statistics of Basement Fungal Indoor-Outdoor Ratios

Moisture/ Mold Indicator	Indoor-Outdoor Fungi Ratio (CFU/m³)								
	TOT			MIF			A/P		
	GM	AM (SD)	Range	GM	AM (SD)	Range	GM	AM (SD)	Range
Musty odor									
Present = 38	1.8	4.8 (9.8)	0.02–58.9	1.7	4.1 (5.1)	0.02–17.9	14.1	89.3 (232.9)	0.15–1213.3
Absent = 20	1.0	3.2 (4.9)	0.05–15.4	1.0	3.7 (6.0)	0.05–20.0	10.1	34.2 (61.5)	0.33–249.3
Efflorescence									
Present = 24	1.7	3.8 (4.5)	0.05–14.4	1.8	4.5 (5.7)	0.05–20.0	18.8	79.8 (167.9)	0.33–807.7
Absent = 34	1.3	4.7 (10.4)	0.02–58.9	1.2	3.6 (5.2)	0.02–17.9	9.5	63.6 (210.8)	0.15–1213.3
Water sources									
Present = 37	2.0[A]	4.4 (5.0)	0.05–15.4	2.1[A]	5.2 (6.2)	0.05–20.0	17.9[B]	96.0 (233.6)	0.15–1213.3
Absent = 20	0.8[A]	4.2 (12.9)	0.02–58.9	0.7[A]	1.9 (2.7)	0.02–11.8	6.7[B]	25.7 (67.7)	0.33–310.1
Visible mold									
Present = 15	2.2	4.3 (4.4)	0.12–13.9	2.4	5.2 (5.9)	0.12–17.9	27.9[B]	49.9 (50.3)	3.63–164.7
Absent = 43	1.2	4.3 (9.5)	0.02–58.9	1.2	3.5 (5.2)	0.02–20.0	9.6[B]	77.4 (222.3)	0.15–1213.3

Notes: GM = geometric mean, AM = arithmetic mean, SD = standard deviation, CFU/m³ = colony forming units per cubic meter; TOT = total; MIF = moisture indicator fungi; A/P = *Aspergillus* and *Penicillium* spp.
[A]The difference between group means is statistically significant ($p < .05$) by independent sample t-test.
[B]The difference between group means approaches statistical significance ($p < .07$) by independent sample t-test

Indoor-Outdoor Fungal Ratios

Homes with water sources in the basement have higher indoor-outdoor fungal ratios than homes without water sources (Table VII). This was found statistically significant in TOT and MIF concentrations and approached significance with A/P ($p = .07$). Basements with visible mold had an indoor-outdoor ratio of A/P three times that of basements without visible mold, and the difference approached statistical significance ($p = .069$). Mean indoor-outdoor ratios of MIF concentrations in homes with moisture/mold indicators ranged from 0.49 to 1.32 in bathrooms and 0.65 to1.17 in bedrooms. Mean indoor-outdoor ratios of MIF in homes with the presence of moisture damage in the bathroom were greater than one (1.32); however, the ratios were not significant when compared with homes without bathroom moisture damage. Mean indoor-outdoor ratios of A/P in homes with the presence of moisture damage and mold in shower were 7.56 and 5.52, respectively; however, they were not statistically significant when compared to homes without these indicators (3.62 and 3.53, respectively). The majority (74%) of indoor-outdoor ratios that exceeded 1 for A/P category occurred at outdoor concentrations <100 CFU/m³ where variability is high, so therefore their interpretation may be questionable.[48]

Regression Analysis

Table VIII provides regression results for all steps using mean MIF concentrations as the dependent variable and for the final step using mean TOT and A/P concentrations. Raw Pearson correlations among the variables are presented first, followed by the predictors of the model. The standardized beta coefficients (β) express the expected indoor concentration change associated with one standard deviation change of the independent variable. For example, the standardized beta coefficient for the association between MIF concentrations and water sources in the basement is .367. This means that for one standard deviation change in water sources in the basement, the predicted indoor MIF log concentration increases by .367 standard deviation.

Basement

Table VIII shows that indoor MIF concentrations are significantly correlated with outdoor concentrations, relative humidity, and moisture indicators in the basement (wall efflorescence, water sources, and visible mold). This same pattern exists for indoor TOT and A/P concentrations. As Table VIII shows, the model was highly predictive of indoor MIF concentrations ($r^2 = .446$, $p = .017$). The same holds true for indoor TOT ($r^2 = .417$, $p = .019$) and indoor A/P ($r^2 = .361$, $p = .022$). When we adjusted for outdoor concentrations and indoor relative humidity, water sources in the basement were found to be significant predictors of indoor TOT, MIF, and A/P concentrations. However, basement water sources and not relative humidity explain 36% of the variance in the model for indoor A/P concentrations.

Bathroom

Indoor fungal concentrations of TOT and MIF were significantly correlated with outdoor fungal concentrations and relative humidity (Table VIII). Visible mold in the shower is significantly correlated with A/P concentrations ($r = .213$, $p = .053$) and approaches significance with TOT ($r = .197$,

TABLE VIII. Stepwise Regression Model to Predict Indoor MIF Concentrations (Log_{10}-Transformed, mg/m^3) in the Basement, Bathroom, and Bedroom; Model C (Final Step Only) to Predict TOT and A/P Concentrations (Log_{10}-Transformed, mg/m^3)

Predictors	Pearson Coefficient MIF	Model A[A] MIF β Coefficient	Model B[B] MIF β Coefficient	Model C[C] MIF β Coefficient	Model C[C] TOT β Coefficient	Model C[C] A/P β Coefficient
Basement						
Outdoor concentrations	.32[E]	.319[E]	.092	.116	.083	.070
Relative humidity	.56[E]	—	.523[E]	.343[E]	.327[E]	.247
Water sources[D]	.53[E]	—	—	.367[E]	.368[E]	.345[E]
Visible mold[D]	.29[E]	—	—	.085	.087	.175
Efflorescence[D]	.37[E]	—	—	−.014	−.010	−026
Basement r^2		0.102	0.324	0.446	0.417	0.361
Bathroom						
Outdoor concentrations	.48[E]	.478[E]	.312[E]	.299[E]	.299[E]	.180
Relative humidity	.51[E]	—	.366[E]	.336[E]	.308[E]	.289
Absent exhaust fan[D]	−.23[E]	—	—	−.083	−.118	.032
Visible mold in shower[D]	.21	—	—	.330	.390	.054
Visible mold in other[D]	.06	—	—	.013	.025	−.053
Visible mold any[D]	.14	—	—	−.270	−.356	.111
Bathroom r^2		0.228	0.335	0.363	0.358	0.229
Bedroom						
Outdoor concentrations	.57[E]	.568[E]	.317[E]	.322[E]	.304[E]	.335[E]
Relative humidity	.64[E]	—	.476[E]	.483[E]	.498[E]	.198
Visible mold[D]	.07	—	—	.032	.038	−010
Visible moisture damage[D]	.09	—	—	.089	.089	.130
Wall-to-wall carpet[D]	.05	—	—	.057	.060	.105
Bedroom r^2		0.323	0.487	0.501	0.507	0.265

[A] Predictor variable in model A = outdoor concentrations.
[B] Predictor variables in model B = outdoor concentrations and relative humidity.
[C] Predictor variables in model C = outdoor concentrations, relative humidity, and moisture indicators.
[D] Dichotomous variables (1 = yes, 0 = no).
[E] p-value < .05.

p = .067) and MIF (r = .21, p = .055) concentrations. Absent external exhaust fan is correlated with indoor TOT (r = −.246, p = .030) and MIF(r = −.225, p = .044) concentrations. When adjusting for outdoor concentrations, indoor relative humidity is a predictor of indoor TOT concentrations (r^2 = .358, p = .007) and MIF concentrations (r^2 = .363, p = .004).

Bedroom

Indoor fungal concentrations of TOT, MIF, and A/P were significantly correlated with outdoor concentrations. In the bedroom, wall-to-wall carpeting is significantly correlated with A/P concentrations (r = .219, p = .045). When we adjusted for outdoor concentrations, indoor relative humidity in the bedroom was found to be a significant predictor of indoor TOT (r^2 = .507, p < .001) and MIF (r^2 = .501, p < .001).

Room Comparison and Basement Water Sources

Regression results show that the "basement water sources" indicator is predictive of fungal concentrations. Figure 1 compares the distribution of the outdoor MIF concentrations and indoor MIF concentrations among the three rooms in the homes with and without basement water sources. Outdoor MIF concentrations were not significantly different between the homes with basement water sources and homes without (p = .27). MIF concentrations were significantly higher in all rooms (basement p < .001; bathroom p = .019; bedroom p < .001) of homes with basement water sources as compared with homes without basement water sources. The MIF concentrations were approximately threefold higher in rooms of homes with basement water sources (bathroom and bedroom means were 910 and 857 CFU/m^3, respectively) as compared with homes without (bathroom and bedroom MIF means were 352 and 238 CFU/m^3, respectively).

DISCUSSION

This is the first article to characterize moisture/mold indicators in residential homes by using a unique fungal

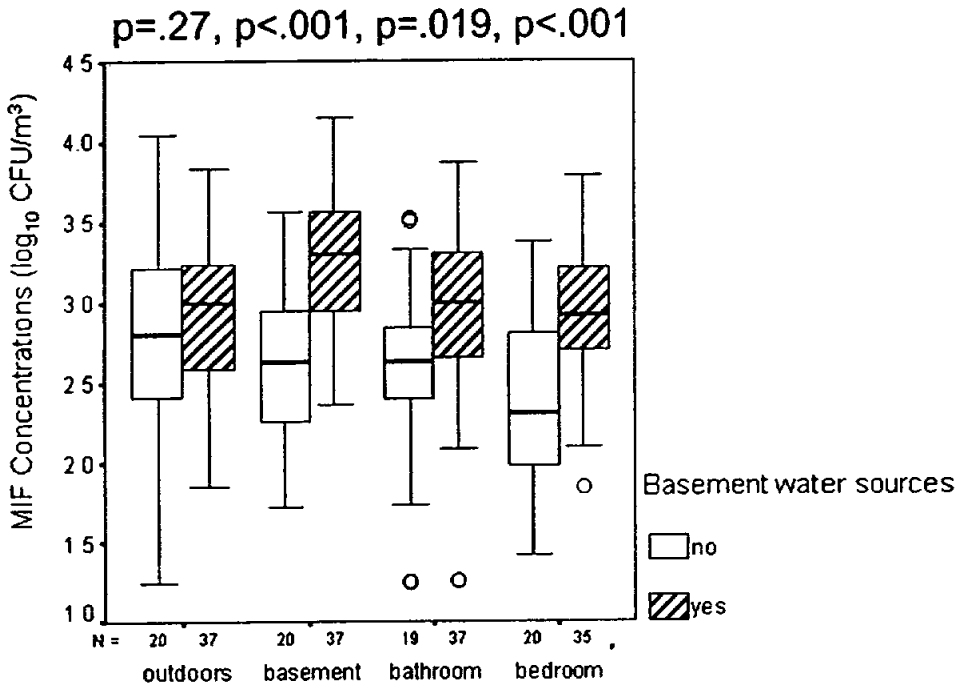


FIGURE 1. Box plots comparing moisture indicator fungi concentrations (log_{10} CFU/m^3) in airborne samples across rooms from homes with and without basement water sources. From the bottom to the top, the box lines in the figure represent 10th, 25th, 50th, 75th, and 90th percentiles, respectively. Circles represent outliers. Outliers are defined as 1.5 box lengths above 75th percentile and 1.5 box lengths below the 25th percentile.

grouping based on characteristics such as water activity and nutrient requirements. Fungi are adaptable to the indoor environment because of the richness of nutrients. The MIF grouping is important because it represents exposure from indoor sources vs. outdoors. *Cladosporium* spp. and *Alternaria alternata* spp., although they are phylloplane fungi and common in outdoor air, were included as moisture indicator fungi because they are commonly found indoors on cold surfaces with condensation, a common phenomenon in the northeastern United States. The MIF grouping may also help with the interpretation of indoor and outdoor fungal ratios of A/P, which was very low outdoors. Grouping species together by their environmental characteristics allows comparison across climates and geographical regions.

It is not feasible to compare airborne fungal concentrations directly across studies for three major reasons: (1) studies were conducted in many parts of the world with different climates (e.g., cold and damp areas in Nordic countries), (2) sampling strategies and protocols varied, and (3) the studies were designed to answer different questions. However, our study and others continue to show that there is wide variability found in airborne fungal concentrations, especially when using cross-sectional study design. Our mean indoor TOT and A/P concentrations were similar to findings in another northeastern study[33] that included the large variability in concentrations. Basement water sources explain 45% of the variability in our model predicting indoor MIF concentrations. Relative humidity also explains some of the variance in most models and may have a secondary impact on mold growth, since a wet substrate has limited drying potential in a high relative humidity environment.

It appears that occupants are able to identify major water leaks in their homes and yet fail to recognize common water problems or sources such as leaky basement windows, poor drainage, cracks in foundations, and static factors, that contribute to moisture entry into the built environment. In our study, occupants reported fewer signs of mold and moisture than inspectors, and there was little agreement found among basement mold/musty odor and basement water problems between SQR and IRC. Trained inspectors observed the following basement water sources that related to the outside structure but were not considered by an occupant: drainage fields that flowed toward the house, below grade with outside rain water draining around foundation, and leaks through the bulkhead doors. The role of surface drainage and the contribution to moisture load in basements was exploratory, and descriptive results indicate that surface drainage quality may be an important factor to consider in future studies. By using the inspector observations in our analysis, reporting bias by subjects was reduced.

Our study shows that rooms with mold and moisture indicators as observed by an inspector have higher fungal

concentrations and physical measurements than rooms without indicators. This is an important finding because the trend in industrial hygiene is to use observational data, rather than air sampling, to document potential mold exposures. We chose to focus on the fungal concentrations of a specific room and see the relationship it has with the moisture/mold indicator in that room—an element of the study design that may account for the significant findings associated with several moisture/mold indicators in our study as compared with the results of others in the literature. For example, homes without an exhaust fan in the bathroom had statistically significantly higher fungal concentrations than homes with a fan. Exhaust fans remove moisture from the bathrooms and therefore control mold growth on surfaces. The presence of operable windows, supported by the building code, may not provide an equivalent solution.

Our study found similar total fungal concentrations in homes with bedrooms with visible mold (geometric mean 892 CFU/m^3) and without mold (561 CFU/m^3) as Dharmage.[30] However, Dharmage tried to quantify the mold surfaces and found significantly greater total fungal propagules in homes with >3 mold surfaces (811 CFU/m^3) in the bedroom than in homes without observed mold (544 CFU/m^3). In our study, it might have been helpful to define or quantify the mold surfaces in a similar manner.

Basements are not usually places where people spend time However, air circulates throughout a home, and our results suggest that basements may provide a source of microbial contamination to living spaces. This may be more of a concern during the heating season for homes in a northern climate. Ren,[11] in a pilot study conducted in 11 Connecticut homes, found higher mean airborne fungal concentrations in basements than in bedrooms and living rooms and outdoor air in most seasons. Indoor basement air differed by the type of fungi and the total fungal concentrations as compared with the other rooms and outdoor air.

Most notably, the highest fungal concentrations were found in the winter in the basement. Ren[11] also found significantly higher concentrations of fungi in the basement of one home with frequent flooding than in the basement of other homes. The measurement of pressure differences between rooms, such as between a bedroom and basement, may be useful for determining how air circulates in a home to indicate potential exposure pathways.

Water sources and mold growth are interrelated variables, the effects of which are difficult to separate from one another. Our findings show that homes with visible mold in the basement have significantly higher A/P concentrations by fourfold than homes without mold. Mean fungal concentrations in basements with mold were two to five times higher than fungal concentrations in bathrooms with mold.

Our findings suggest that the indicator "mold in basement" may represent an important source of exposure throughout the dwelling that has largely been ignored in the literature. In most houses, warm air rises to the upper portions of a building envelope to induce a "stack effect" or "chimney effect." This stack effect[50] creates a negative pressure in the basement. This tends to draw moist exterior air in through cracks or openings in the foundation and may facilitate fungal growth and subsequent migration of fungal material throughout the house. More research is needed to investigate the relationship among a "wet" basement, other occupied spaces in the home, and health symptoms.

Indoor-outdoor ratios were calculated to determine whether microbial amplification occurs in the homes. Most notably, mean indoor-outdoor fungal ratios for homes with water sources were significantly higher than homes without. Our study provides additional data to explore the feasibility of using indoor and outdoor fungal ratios in exposure assessment. The use of indoor-outdoor ratios in an exposure assessment is still limited. This is most evident for A/P concentrations, whereby the indoor and outdoor geometric mean ratios of homes with an indicator in the basement ranged from 14–28 CFU/m^3 as compared with homes without indicators (7–10 CFU/m^3). The ratios exceeding 1.0 occurred at outdoor concentrations of < 100 CFU/ m^3. Using data from an office building with mechanical ventilation, Burge[48] reported that the importance of indoor and outdoor ratios exceeding 1.0 at outdoor concentrations <100 should be interpreted with caution.

This study was conducted in the state of Connecticut, and the small sample size and the voluntary nature of participation may somewhat limit the generalization of the findings. Nevertheless, the results are consistent with what is expected from environmental microbiology. In addition to geography, season plays an important role when interpreting the results of fungal sampling. The study homes were sampled throughout the year, with a large percentage of homes visited in the spring and summer. Ideally, sampling homes in one season would reduce some of the variability of airborne fungal concentrations and physical measurements. In our study, as a way to account for seasonality, indoor fungal concentrations were adjusted by outdoor concentrations and relative humidity in a regression analysis. Indoor and outdoor fungal concentration ratios were also presented as a way to account for seasonality.

This study was carried out in the late 1990s, and the current trend for identifying a "moldy or "moisture problem dwelling" may be shifting away from relying on airborne fungal sampling for exposure assessment. Most recently, Haverinen[2] has shown that an exposure classification system using indicators is more predictive of health symptoms (with an observed dose-response relationship) when the exposure model accounts for the amount of moisture damage and estimated severity. Due to the complexity and technical inability to document fungal exposures that may occur with the method of air sampling, this kind of classification system is promising.

CONCLUSIONS

This study characterized several moisture and mold indicators in residential dwellings using conventional sampling

methods and presents airborne fungal concentrations in three different fungal categories. The MIF grouping is considered an alternative predictor variable to total or a single dominant culturable species in future analyses regarding health risks and moisture or mold. Basement water sources are an important moisture/mold indicator in residential dwellings for epidemiologists to utilize in exposure assessments. Educating the homeowner about less obvious water sources or moisture intrusion, and the importance for their timely repair, is needed to prevent microbial contamination in basements and thus to other occupied areas. Longitudinal studies are necessary to better understand the relationships between environmental characteristics, microbial growth, and health risks.

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CERTIFICATE OF SERVICE

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